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(57) Abstract

The present invention relates to variants of Penicillin Binding Proteins (PBP), which proteins are involved in bacterial peptidoglycan biosynthesis. Disclosed are also DNA molecules coding for the said PBP variants, as well as vectors and cells harbouring such DNA molecules. The invention is also related to processes for assaying and designing therapeutically useful compounds which have high affinity to PBP, which processes utilize the said PBP variants.

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NOVEL POLYPEPTIDES

TECHNICAL FIELD

The present invention relates to variants of Penicillin Binding Proteins (PBP), which proteins are involved in bacterial peptidoglycan biosynthesis. Disclosed are also DNA molecules coding for the said PBP variants, as well as vectors and cells harbouring such DNA molecules. The invention is also related to processes for assaying and designing therapeutically useful compounds which have high affinity to PBP, which processes utilize the said PBP variants.

BACKGROUND ART

Bacteria and most other unicellular organisms possess a cell wall, which comprises a cross-linked polysaccharide-peptide complex called peptidoglycan. Peptidoglycan biosynthesis consists of three stages: (1) synthesis of precursors (sugar nucleotides) in the cytosol, (2) precursor transfer across the membrane and formation of the polysaccharide chain, and (3) cross-linking of individual peptidoglycan strands in the cell wall.

In the latter stage of peptidoglycan biosynthesis, new bonds must be made between nascent glycan strands and existing peptidoglycan. The newly synthesized chains are about 10 disaccharides long and are extended by transglycosylase enzymes to a final glycan strand of between 100 and 150 disaccharide units. The peptidoglycan is crosslinked by the action of transpeptidases which link the terminal D-ala of one glycan strand to a free ε-amino group on a diaminopimelic acid residue on an adjacent region.

A number of antibiotics inhibit bacterial growth by interfering with the formation of the peptidoglycan layer. The cross-linking reaction is the

target for action of two important classes of such antibiotics, the penicillins and the cephalosporins. Penicillin is thought to react irreversibly with the transpeptidase that catalyses cross-linking.

The penicillin interactive proteins fall into three groups: the β-lactamases, the Low Molecular Weight-Penicillin Binding Proteins (PBPs), which mainly include the carboxypeptidases, and the High Molecular Weight-Penicillin Binding Proteins. Penicillin Binding Proteins are those enzymes which have been shown to bind radiolabelled penicillin G. In *Escherichia coli* such proteins are called e.g. PBP 1A and PBP 1B, both belonging to the class High Molecular Weight-PBPs. PBP 1A and 1B, which are known to be membrane bound proteins, maintain cell integrity and control peptidoglycan side wall extension during growth. Inactivation of either PBP 1A or PBP 1B can be tolerated by the bacteria while the deletion of both the genes, designated *pon*A and *pon*B, is lethal (Yousif et al., 1985).

PBP 1B is known to be a bifunctional enzyme possessing both transpeptidase and transglycosylase activity (Ishino et al., 1980). PBP 1A is believed to be bifunctional since it can substitute for PBP 1B. The β-lactam antibiotics, such as penicillin, inhibit only the transpeptidase activity of these proteins.

The transglycosylase reaction is inhibited by e.g. moenomycin, which is a phosphoglycolipid used as a growth promoter in animal nutrition and which has been shown to possess broad spectrum bactericidal activity. The enzyme transglycosylase has been shown to be present in *Escherichia coli, Staphylococcus aureus, Bacillus megaterium* and *Bacillus subtilis*. This suggests that interference of peptidoglycan biosynthesis by inhibition of transglycosylase could be a lethal event in all clinically important pathogens.

The putative transglycosylase domain of PBP 1B has been assigned to the N-terminal 478 amino acids (Nakagawa et al., 1987). This regions includes three conserved stretches of amino acids between the N-terminal half of both PBP 1A and 1B and could represent residues involved in the transglycosylase activity.

Preparation of Penicillin Binding Protein 2A from *Staphylococcus aureus* is disclosed in EP-A-0505151.

DISCLOSURE OF THE INVENTION

There is a growing number of reports of bacteria which are resistant to antibiotics. There is consequently a need for new compounds which inhibit bacterial growth by means of binding Penicillin Binding Proteins. The present invention provides PBP variants which facilitate processes for assaying and designing therapeutically useful compounds which have high affinity to PBPs.

Accordingly, it is an object of the invention to provide polypeptides which are water-soluble active derivatives of bacterial bifunctional Penicillin Binding Proteins, said Penicillin Binding Proteins being bound to the cell membrane when expressed in a bacterial cell and being capable of exhibiting both transglycosylase and transpeptidase activities and said derivatives lacking a membrane anchoring sequence but retaining the capability to exhibit one or both of said enzymic activities. The "bacterial cell" mentioned above is preferably an *Escherichia coli* cell or a *Streptococcus pneumoniae* cell.

The soluble PBP variants according to the invention retains transglycosylase activity, indicating that soluble variants of PBP, devoid of membrane anchoring sequences, can recognize lipid linked substrate and polymerise the disaccharide into repeating units. It can thus be assumed

that other analogues of PBP lacking residues involved in membrane attachment would be enzymatically functional.

Molecules interacting with the penicillin interactive region of soluble PBP variants could be assumed to be capable of interacting identically with wild-type PBPs. Consequently, the soluble PBP variants according to the invention can be used for identifying compounds which are interacting with wild-type Penicillin Binding Proteins.

It is furthermore well known that membrane-bound proteins are very difficult to crystallize. The soluble enzymatically active PBP variants can be used for crystallisation and will thereby facilitate a rational design, based on X-ray crystallography, of therapeutic compounds inhibiting High Molecular Weight-PBPs.

A further object of the invention is to provide polypeptides which are truncated water-soluble derivatives of bacterial bifunctional Penicillin Binding Proteins, said Penicillin Binding Proteins being bound to the cell membrane when expressed in a bacterial cell and being capable of exhibiting both transglycosylase and transpeptidase activities and said derivatives lacking the membrane anchoring sequence but retaining the capability to exhibit the transglycosylase activity. The "bacterial cell" mentioned above is preferably an *Escherichia coli* cell.

Alignment of amino acid sequences of High Molecular Weight-Penicillin Binding Proteins, and the compilation of the motifs involved in the penicillin binding of β-lactamases and carboxypeptidase, have suggested the C-terminal half of PBP 1A and 1B to be the functional domain of the transpeptidase activity and includes the penicillin binding domain. In addition, Nakagawa et al. (1987) showed that a truncated *pon*B gene encoding the N-terminal 478 amino acids of PBP 1B is capable of the transglycosylase reaction.

On the basis of these findings, it has been suggested that the high molecular weight PBP 1A and 1B proteins are two domain-proteins, with the N-terminal half forming the transglycosylase domain and the C-terminal half the transpeptidase domain. The two domains have been predicted by computer analysis to be joined by a linker or hinge region which does not structurally or enzymatically contribute to the function of the protein. The linker region of *E.coli* PBP 1B has been predicted to be from position 545-559 while that for *E.coli* PBP 1A around position 501.

The monofunctional truncated variants of PBP according to the invention will, when used in x-ray crystallography, facilitate obtaining structural information of the transglycosylase domain of penicillin binding proteins. In addition, the reduced size of the monofunctional variant will facilitate crystallization.

In a preferred form, a water-soluble polypeptide according to the invention has an amino acid sequence which is identical to SEQ ID NO: 2, 4, 6, 12 or 13 in the Sequence Listing.

The observation that deletion of the *pon*A and *pon*B genes is lethal (Yousif et al., 1985) does not address the question of essentiality of the transglycosylase activity of the encoded PBP 1A proteins, since the deletion results in the loss of both transglycosylation and transpeptidation activities. In addition, this experiment does not address the possibility that the transglycosylase enzyme activity can be contributed by a Penicillin Binding Protein other than PBP 1A or PBP 1B. It is also possible that hitherto undescribed Penicillin Binding Proteins and/or other proteins that contribute to the transglycosylase activity exist.

Alignment of the amino acids forming the putative transglycosylase domain of PBP 1A and 1B reveals three stretches of 9 out of 12 (Region 1), 9/10 (Region 2) and 8/10 (Region 3) amino acids identical within the

N-terminal half of these two proteins (Broome-Smith et al., 1985) (Fig. 14). The same 3 regions are identically conserved among two other recently described protein sequences; *Streptococcus pneumoniae* PBP 1A (Martin et al., 1992) and a 94 kDa protein from *Haemophilus influenzae* (Tomb et al., 1991). The conservation of these residues in such diverse species suggests their critical requirement in either maintaining structural aspects of the protein, or in the transglycosylation reaction itself.

The overlapping functional transglycosylase and transpeptidase activities of PBP 1A and 1B also suggests conservation of the catalytic centres and that molecules designed to interact with the catalytic centre of PBP 1A would be reactive also with PBP 1B.

The functional transglycosylase activity of the expressed protein can be studied either in a direct *in vitro* assay using appropriate substrates, or in an assay measuring the ability of the protein to complement the deletion of the corresponding genes in the chromosome. It has been shown that a plasmid with a gene encoding the wild type product (PBP 1A or PBP 1B) is capable of maintaining the viability of the *E.coli* cell (Yousif et al., 1985). This trans-complementation technique can be utilized to assess the functional nature of the mutant gene(s) encoding PBPs with mutations inactivating one of the enzymic (transglycosylation or transpeptidation) functions. The ability of such mutant products to complement in trans the deletion of the chromosomal *pon*A and *pon*B genes would define the essential requirement of the individual enzymic functions.

There is consequently a need for research tools which will make it possible to study the effects of specific inactivation of the transglycosylase activity of Penicillin Binding Proteins.

Consequently, a further aspect of the invention is a polypeptide which is a transglycosylase deficient derivative of a bacterial bifunctional penicillin

binding protein, said penicillin binding protein being bound to the cell membrane when expressed in a bacterial cell and being capable of exhibiting both transglycosylase and transpeptidase activities and said derivative lacking the capability to exhibit transglycosylase activity but retaining the capability to exhibit transpeptidase activity. The "bacterial cell" mentioned above is preferably an *Escherichia coli* cell.

The transglycosylase deficient PBP variants can advantageously be used in X-ray crystallography for the purpose of obtaining structural information of the activity sites of PBPs. Structural analysis of crystal form of soluble transglycosylase deficient PBP variants could allow delineation of the catalytic region and facilitate the design of molecules capable of specifically inhibiting the transglycosylase activity.

In a preferred form, the transglycosylase deficient polypeptide according to invention is a polypeptide which is lacking transglycosylase activity because of a mutation or deletion in the second conserved region of the gene coding for said polypeptide.

In a further preferred form, the transglycosylase deficient polypeptide according to the invention has an amino acid sequence which is identical to SEQ ID NO: 7, 8, 9, or 10 in the Sequence Listing.

The conventional purification procedure employed for the enrichment of penicillin binding proteins has been the use of a "penicillin" affinity. The binding of the protein to penicillin is covalent and requires harsh conditions to elute the bound protein. This may lead to a certain degree of inactivation of the enzymic activity of the protein. There is consequently a need for alternate affinity matrices for the efficient purification of the proteins.

Included in the invention is consequently a polypeptide comprising (a) a first polypeptide which is a PBP variant according to the invention; and (b) an additional polypeptide which allows binding to an affinity matrix; there being a cleavage site between said polypeptides.

The "additional polypeptide" mentioned above can preferably be glutathione-S-transferase or a polypeptide substantially similar to glutathione-S-transferase. Such an additional polypeptide will enable rapid purification of the protein using Glutathione Sepharose® affinity matrix. In another preferred form, the additional polypeptide is a polypeptide rich in histidine residues, which residues will confer on the protein the ability to bind to an Ni affinity column. The additional polypeptide can be fused either to the N-terminus or the C-terminus of the soluble/membrane bound PBP.

The ability of the fusion proteins to bind to an affinity matrix allows immobilisation of the protein. Such immobilised proteins can be used for analysis of competitive binding of different ligands to the bound active protein, and thus for screening of compounds binding to the enzymic domain of interest.

The polypeptides according to the invention are not to be limited strictly to any one of the sequences shown in the Sequence Listing. Rather the invention encompasses polypeptides carrying modifications like substitutions, small deletions, insertions or inversions, which polypeptides nevertheless have substantially the biochemical activities of the PBP variants which amino acid sequence is disclosed in the Sequence Listing. Included in the invention are consequently also polypeptides, the amino acid sequence of which is at least 90% homologous, preferably at least 95% homologous, with the amino acid sequence of any of the PBP variants according to the invention.

A further object of the invention is to provide isolated and purified DNA molecules which have nucleotide sequences coding for any one of the PBP variants according to the invention.

In a preferred form of the invention, the said DNA molecules have nucleotide sequences identical to SEQ ID NO: 1, 3 or 5 in the Sequence Listing. However, the DNA molecules according to the invention are not to be limited strictly to any of the sequences shown in the Sequence Listing. Rather the invention encompasses DNA molecules carrying modifications like substitutions, small deletions, insertions or inversions, which nevertheless encode proteins having substantially the biochemical activities of the PBP variants according to the invention.

Included in the invention is also a DNA molecule which nucleotide sequence is degenerate, because of the genetic code, to the said nucleotide sequence coding for a PBP variant according to the invention. The natural degeneracy of the genetic code is well known in the art. It will thus be appreciated that the DNA sequences shown in the Sequence Listing are only examples within a large but definite group of DNA sequences which will encode the PBP variants which amino acid sequences are shown in the Sequence Listing.

A further aspect of the invention is a replicable expression vector which carries and is capable of mediating the expression of a DNA molecule according to the invention. In the present context the term "replicable" means that the vector is able to replicate in a given type of host cell into which is has been introduced. Examples of vectors are viruses such as bacteriophages, cosmids, plasmids and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art. A vector according to the invention can preferably be one of the plasmids listed in Table 1 below.

Included in the invention is also a host cell harbouring a vector according to the invention. Such a host cell can be a prokaryotic cell, a unicellular eukaryotic cell or a cell derived from a multicellular organism. The host cell can thus e.g. be a bacterial, yeast or mammalian cell. The methods employed to effect introduction of the vector into the host cell are well-known to a person familiar with recombinant DNA methods.

A further aspect of the invention is a process for production of a polypeptide which is a derivative of penicillin binding protein, comprising growing a host cell according to the invention in or on a culture medium for expression of the polypeptide and optionally recovering the polypeptide. An appropriate host cell may be any of the cell types mentioned above, and the medium used to grow the cells may be any conventional medium suitable for the purpose.

The High Molecular Weight-Penicillin Binding Proteins have been shown to be anchored to the membrane, but the majority of the protein is within the periplasmic space of the cell (Edelman et al. 1987). Thus PBP derivatives, devoid of the membrane signal / anchoring sequences, are forced to fold into their native state in a heterologous environment, namely the cytosol. This often leads to misfolding, and the majority of the expressed protein aggregates into an inactive form referred to as inclusion bodies.

It has now surprisingly been found that high yields of an active water-soluble PBP variant can be obtained by regulated transcription of the gene encoding the said PBP variant. Such regulated transcription involves (i) using a suboptimal concentration of the inducer isopropyl thiogalactoside (IPTG); and (ii) culturing the cells expressing the PBP variant at reduced temperature. A cumulative effect of these factors contributes to the overall recovery of the active soluble protein. Consequently, lower rates of expression, achieved through the mentioned combination of (i) sub-optimal

de-repression of promoter systems and (ii) increased generation time by lowering of the temperature of cultivation, will enhance the solubility of proteins lacking the membrane anchoring segment.

A further important aspect of the invention is a process for the production of a water soluble polypeptide according to the invention which comprises culturing *Escherichia coli* cells harbouring an expression vector wherein a DNA coding sequence for said polypeptide is under the control of an isopropyl thiogalactoside (IPTG) - inducible promoter, said culturing being carried out in the presence of a sub-optimal concentration of IPTG for induction of the said promoter and at a temperature in the range of 20 to 24°C, preferably 22°C. The concentration of IPTG can preferably be approximately 0.01 mM.

In the case of expression of *pon*Adel23, a gene encoding a PBP variant according to the invention, such regulated transcription by (i) controlled de-repression of the T7 promoter by using sub-optimal concentration of the inducer IPTG and (ii) reducing the growth rate by culturing at 22°C, resulted in yields of the active protein which reached nearly 50% of the total induced protein of interest. The growth and induction conditions were critical for the efficient recovery of the soluble protein, as growth at higher temperatures or induction with higher concentrations of IPTG resulted in the majority of the protein becoming inactive and forming inclusion bodies.

I will be appreciated that this method for controlled expression is applicable to other inducible promoter systems, e.g. the tac system, where the inducer is IPTG and the host is a lac Y negative host.

A route to obtain relevant structural information on the active site configuration of an enzyme is the production and characterisation of monoclonal antibodies capable of inhibiting the enzymic reaction. The

antibodies inhibiting the activity represent molecules which block or compete with the substrate for entry into the active site pocket, or can represent molecules which can prevent structural transitions required for catalytic activity. In either case, these antibodies can be used as a tool to quantitate interaction of the target enzyme with binding of radiolabelled inhibitory compounds to judge the affinity of interaction provided the affinity of the inhibiting antibody is known. A further use of mapping the epitopes recognised by the inhibitory antibodies is the ability to delineate residues forming the active site.

Consequently, a further aspect of the invention is a method of identifying an antibody capable of binding a bacterial bifunctional penicillin binding protein which includes the step of employing a polypeptide according to the invention in an antibody binding assay and selecting antibodies that bind to the polypeptide.

Also included in the invention are monoclonal antibodies directed to a PBP variant according to the invention. Such a monoclonal antibody is prepared using known hybridoma technology by fusing antibody-producing B-cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody.

Another aspect of the invention is a method of assaying for compounds which bind to a penicillin binding protein, said method comprising (a) contacting a polypeptide which is a PBP variant according to the invention with a compound to be investigated; and (b) detecting whether said compound binds to the said PBP variant.

For example, a method of assaying for compounds which bind to a penicillin binding protein can comprise (a) culturing host cells according to the invention; (b) lysing the said cells and isolating the crude cell extract; (c) exposing the said cell extract to potential inhibitors of a penicillin

binding protein; (d) introducing an agent, known to bind a penicillin binding protein, to the said cell extract; (e) removing the unbound fraction of said agent; and (f) assaying the presence of said agent remaining in the cell extract.

Another method of assaying for compounds which bind to a penicillin binding protein could comprise (a) exposing a polypeptide which is a PBP variant according to the invention, immobilised on a solid support, to a potential inhibitor of a penicillin binding protein; (b) exposing an agent, known to bind a penicillin binding protein, to the immobilised polypeptide; (c) removing the unbound fraction of said agent; and (d) assaying the presence of said agent bound to the immobilised polypeptide.

In a preferred form, the said method is a method of assaying for compounds which bind to the transglycosylase domain of a penicillin binding protein, said method comprising (a) exposing the transglycosylase domain of a polypeptide according to the invention, with the proviso that the polypeptide is not a transglycosylase deficient PBP variant, said polypeptide being immobilised on a solid support, to a potential inhibitor of the transglycosylase activity of a penicillin binding protein; (b) exposing an agent, known to bind the transglycosylase domain of a penicillin binding protein, to the immobilised polypeptide; (c) removing the unbound fraction of said agent; and (d) assaying the presence of said agent bound to the immobilised polypeptide.

Antibodies specific for transpeptidase can be immobilised on a BIAcore sensor chip surface. The BIAcore system, wherein "BIA" stands for "Biospecific Interaction Analysis", is available from Pharmacia Biosensor, Sweden. Protein binding to the immobilised antibody is detected by the output RU-signal. Screening for TP inhibitors will be possible by a competitive assay wherein soluble protein is preincubated with test compounds. Binding of a test compound to the protein will result in a

decrease in protein binding to TP specific antibody. In the same way, monoclonal antibodies specific for transglycosylase can be used in screening for TG inhibitors.

In a similar way, ampicillin or modified moenomycin can be coupled to the surface and used in an indirect competitive assay whereby protein is preincubated with test ligand prior to introduction in the BIAcore.

Consequently, yet another method of assaying for compounds which bind to a penicillin binding protein could comprise (a) exposing a polypeptide which is a PBP variant according to the invention to a potential inhibitor of a penicillin binding protein; (b) exposing the polypeptide to an agent, known to bind a penicillin binding protein, which agent is immobilised on a solid support; and (c) assaying the presence of polypeptide bound to the immobilised agent.

In a preferred form, the said method is a method of assaying for compounds which bind to the transglycosylase domain of a penicillin binding protein, said method comprising (a) exposing the transglycosylase domain of a polypeptide according to the invention, with the proviso that the polypeptide is not a transglycosylase deficient PBP variant, to a potential inhibitor of a penicillin binding protein; (b) exposing the said polypeptide to an agent, known to bind to the transglycosylase domain of a penicillin binding protein, which agent is immobilised on a solid support; and (c) assaying the presence of polypeptide bound to the immobilised agent.

The "agent known to bind a penicillin binding protein" referred to above can e.g. be a monoclonal antibody or a labelled antibiotic compound such as [^{3}H]ampicillin.

A further aspect of the invention is a method of determining the protein structure of a penicillin binding protein, characterized in that a polypeptide which is a PBP variant according to the invention is utilized in X-ray crystallography.

Some of the features of the preferred PBP variants according to the invention are summarised in Table 1 below. The plasmids listed in the Table have been deposited under the Budapest Treaty at the National Collection of Industrial and Marine Bacteria Limited (NCIMB), Aberdeen, Scotland, UK. The date of deposit is 28 June 1994.

TABLE 1

Example no.	Features	Plasmid (pARC)	Deposit no. (NCIMB)	Fig.	SEQ ID NO:
Soluble variants					
1.1	*E.coli* PBP 1A with aa 1-23 deleted	0558	40666	3	1, 2
2.1	*E.coli* PBP 1B with aa 65-87 deleted	0559	40667	9	3, 4
3.1	*S.pneumoniae* PBP 1A with aa 1-38 deleted	0512	40665	12	5, 6
Transglycosylase deficient variants					
4.1	*E.coli* PBP 1B with glutamines 270-271 substituted to alanines	0438	40661		7
	E.coli PBP 1B with glutamines 270-271 substituted to leucines	0468	40662		8
	E.coli PBP 1B with aa 264-271 deleted	0469	40663		9
4.2	*E.coli* PBP 1A with glutamines 123-124 substituted to alanines	0571	40668	19	10
Truncated variants					
5.1	aa 1-553 of *E.coli* PBP 1B	0592	40669	21	11
	aa 1-553 of *E.coli* PBP 1B, with aa 65-87 deleted	0593	40670	22	12
5.2	aa 210-368 of *E.coli* PBP 1B	0392	40659	23	13
Fusion proteins					
6.1	*E.coli* PBP 1A with 23 aa deletion, ligated to glutathione-S-transferase	0499	40664	24	
6.2	*E.coli* PBP 1A with 23 aa deletion, ligated to histidine stretch	0400	40660	25	

EXAMPLES OF THE INVENTION

In the following examples, the terms "standard protocols" and "standard procedures" are to be understood as protocols and procedures found in an ordinary laboratory manual such as the one by Sambrook, Fritsch and Maniatis (1989).

EXAMPLE 1

1.1. Construction of gene encoding soluble form of *E.coli* PBP 1A

The possible amino acid residues involved in the membrane anchoring region of PBP 1A was deduced following the computer program described by Kyte & Dolittle (1982). The predicted hydrophobicity of the N-terminal 60 amino acid is shown in Fig. 1. Based on this hydrophobicity profile, it was predicted that the N-terminal 23 amino acids were strongly implicated to contribute to the membrane anchoring domain of the protein, but may not entirely encompass the membrane anchoring domain. This region was then putatively designated as the region involved in "membrane anchoring".

The plasmid pBS98, harbouring the native *ponA* gene (encoding wild type PBP 1A), was obtained from Prof. B.S. Spratt, Microbial Genetics Group, School of Biological Sciences, University of Sussex, Brighton, UK. The construction of pBS98 is described in Broome-Smith et al. (1985). Plasmid DNA from cells harbouring pBS98 was made following standard protocols.

Oligonucleotide primers for use in the polymerase chain reaction (PCR) were synthesized in Applied Biosystems Model 380 A. The 5'-oligonucleotide primer used was TG-82:

```
            NcoI
             ↓
5'-TCG ACC ATG GGC CTA TAC CGC TAC ATC G-3'
           M   G   L   Y   R   Y   I
           23  24  25  26  27  28  29  (Amino acid No.)
```

TG-82 incorporates the following characteristics: (1) it allows construction of mutant *ponA* gene whose encoded product would have the 24th amino acid (glycine) of the wild type PBP 1A as the second amino acid of the expressed mutant protein; and (2) it introduces DNA sequences recognized by the restriction enzyme *Nco*I. This introduces the codon ATG which corresponds to the first amino acid of the mutant PBP 1A when expressed in suitable systems.

The 3'-oligonucleotide primer used was TG-64:

```
5'-CGC GGA TCC GAA TCA CAA CAA TTC CTG TGC-3'
        ↑
      BamHI
```

TG-64 has the following characteristics: (1) it introduces a termination codon following the 850th amino acid of the structural protein of PBP 1A; (2) it introduces a site for the restriction enzyme *Bam*HI to facilitate cloning into suitable expression vectors.

Using these primers, PCR was carried out using pBS98 DNA as template following standard protocols. A DNA fragment of approximately 2.5 kb was amplified. The fragment was digested with the restriction enzyme *Nco*I followed by digestion with *Bam*HI. This 2.5 kb *Nco*I - *Bam*HI DNA fragment was then ligated to the vector pBR329 (Covarrubias et al., 1982) previously cut with *Nco*I and *Bam*HI. Ligation of the two DNA fragments were carried out using standard protocols and the ligation mixture transformed into *E.coli* DH 5α. The transformed cells were plated on LB agar plates with 50 µg/ml ampicillin. Following overnight incubation at 37°C, individual ampicillin resistant colonies were tested for their

tetracycline sensitivity as insertion into the *Nco*I - *Bam*HI region renders the plasmid chloramphenicol and tetracycline sensitive. A recombinant plasmid bearing the 2.5 kb insert was designated pARC0488.

The *Nco*I - *Bam*HI 2.5 kb DNA fragment was released from pARC0488 and ligated to *Nco*I - *Bam*HI cleaved and purified pARC038 (Fig. 2). The plasmid pARC038 is a derivative of pET11d (Studier et al., 1990) in which the *Eco*RI and *Pst*I sites were made blunt ended with T4 exonuclease and the *Eco*RI - *Pst*I 0.75 kb DNA fragment replaced with a blunt ended kanamycin resistance cartridge (Pharmacia Biochemicals). The ligation mixture was transformed into competent cells of *E.coli* BL 26 (DE3). The transformation mix was plated on LB agar with 50 µg/ml kanamycin. Mini-prep plasmid DNA was made from several kanamycin resistant colonies and screened by restriction endonuclease mapping using standard procedures.

One of the colonies harbouring plasmid with expected structure (Fig. 3) was labelled pARC0558 (NCIMB 40666). The DNA sequence of the mutant *pon*A gene labelled as *pon*Adel23 is shown as SEQ ID NO: 1. The amino acid sequence of the soluble PBP 1Adel23 is shown as SEQ ID NO: 2.

1.2. Expression of *pon*Adel23

E.coli BL 26 (DE3) cells (obtained from Dr. J.J. Dunn, Biology Dept., Brookhaven National Lab., Long Island, NY, USA) harbouring pARC0558 were grown in LB with 50 µg/ml kanamycin till an O.D. at 600 nm of 0.6 and induced with 0.01 mM isopropyl thiogalactoside (IPTG) for 6 hours.

Following 6 hours of induction, cells were harvested and broken by passing through a French press. After centrifugation at low speed to remove unbroken cells and debris, the cytosolic (soluble) fraction was obtained by either of the following two methods: (1) following a procedure

described Page et al. (1982) in which the pellet, membrane and soluble proteins are separated by sucrose gradient centrifugation; or (2) by spinning the obtained supernatant at 200,000 x g for 90 minutes, whereafter the supernatant obtained is taken as the cytosolic / soluble protein fraction.

1.3. Penicillin binding of expressed PBP 1Adel23

The obtained cytosolic fraction was tested for the presence of mutant PBP 1A by following the method of Rojo et al. (1984). This procedure involves using [^{125}I]cephradine as the labelled penicillin as it is specific for PBP 1A. Mutant PBP 1Adel23 capable of binding the labelled cephradine could be demonstrated in the cytosolic fraction. Approximately 50% of the expressed mutant protein fractionated as a soluble protein, while the remaining 50% fractionated into the inclusion body and/or into the membrane associated fractions. Consequently, enhanced levels of active mutant PBP 1Adel23 were obtained since the cells were induced with sub-optimal concentration of IPTG and the since cultures were grown at 22°C. The penicillin binding profile of the soluble PBP 1Adel23 is shown in Fig. 4.

1.4. Purification of soluble PBP 1Adel23

The cell pellet of *E.coli* BL26 (DE3) / pARC0558 obtained following 6 hours of induction at 22°C was washed twice with buffer A (30 mM Tris-Cl, pH 8.0; 10 mM EDTA; 10 µg/ml leupeptin; 10 µg/ml aprotinin; 5 mM DTT) and resuspended in the same buffer. The cell suspension was passed through a French press at 1200 psi. The lysate was spun at 10,000 rpm for 10 minutes and the obtained supernatant centrifuged at 200,000 x g for 45 minutes. The obtained supernatant was then adjusted to 30% saturation with ammonium sulphate. The mixture was centrifuged at 12,000 rpm for 10 min and the pellet resuspended in buffer A containing 1 M NaCl. The dissolved pellet was then treated with Cephradine-Affigel 10 matrix.

Cephradine was conjugated to Affigel 10 following the instructions of the manufacturers (Biorad Laboratories, USA). The soluble PBP 1Adel23 containing fraction, dissolved in buffer A containing 1 M NaCl, was incubated 16 hrs at 4°C. with cephradine-affigel 10 beads. The beads were then washed with Buffer A containing 1 M NaCl until the absorbance at 280 nm was nearly zero. Elution of PBP 1Adel23 was monitored by assaying for penicillin binding activity in the wash. This activity was measured using [^{125}I]cephradine prepared as described in Rojo et al. (1984). Bound PBP 1Adel23 was eluted from the beads using 1 M hydroxylamine (pH 8.5) at 25°C for 120 minutes. This fraction was concentrated by ultrafiltration using YM 30 filters (Amicon, USA) in Buffer A with 0.25 M NaCl. The ultrafiltration also resulted in the removal of hydroxylamine. The purified fraction containing >85% of the protein species corresponding to PBP 1Adel23 showed both penicillin binding and transglycosylase enzyme activities. The protein profile as seen by Coomassie Brilliant Blue staining and the [^{125}I]cephradine / penicillin binding profile of the different fractions, obtained during the various stages of purification, are shown in Fig. 5. The N-terminal amino acid sequence of the soluble PBP 1Adel23 was confirmed by sequencing the purified protein.

1.5. Transglycosylase activity of soluble PBP 1Adel23

The transglycosylase activity of the soluble PBP 1Adel23 protein was measured using essentially the method described by Ishino et al. (1980). The substrate for the detection of the enzymic activity were essentially prepared and purified following the protocols described by Heijenoort et al. (1992). The concentration dependent transglycosylase activity of PBP 1Adel23 measured as the amount of peptidoglycan formed was compared to the amounts of peptidoglycan formed by different concentrations of the membrane bound form of native PBP 1A. As seen in Fig. 6, the peptidoglycan polymerizing efficiency of the mutant soluble PBP 1Adel23

was nearly identical to the enzymic activity of the membrane bound form of the protein.

It has consequently been found that the elimination of the 23 amino acid residue stretch does not interfere with the ability of the protein to assume its native structure capable of both the enzymatic activities, i.e. the transglycosylase and the transpeptidase activities.

EXAMPLE 2

2.1. Construction of gene encoding soluble form of *E.coli* PBP 1B

The *pon*B gene encoding PBP 1B was obtained on a plasmid pBS96 from Prof. B.S. Spratt, Microbial Genetics Group, School of Biological Sciences, University of Sussex, Brighton, UK. The construction of pBS96, as well as the nucleotide sequence of the wild-type *pon*B gene and the derived amino acid sequence, are described in Broome-Smith et al. (1985).

The hydropathy plot of the N-terminal approximately 150 amino acids as derived using the method of Kyte and Doolittle (1982) is shown in Fig. 7. Analysis of the hydropathicity plot indicated that the amino acids at positions 65 to 87 of the PBP 1B sequence contributed largely to the hydrophobicity of the N-terminus and can be putatively assigned to be the membrane anchoring domain of the protein. In addition, β-lactamase studies of Edelman et al. (1987) had indicated that amino acids C-terminal to amino acid position 87 were present in the periplasmic space of the *E.coli* cell and that amino acids N-terminal to position 65 of PBP 1B were within the cytoplasm of the cell.

The strategy employed to construct a mutant *pon*B gene encoding a soluble form of PBP 1B is shown in Fig. 8. Initially a DNA fragment of approximately 200 bp of the 5'-end of the *pon*B gene was amplified by

PCR, from the *pon*B gene on the plasmid pBS96 (Broome-Smith et al., 1985). The oligonucleotide primers used were 5'-primer TG-77 (5'-GAA AAA CCA TGG CCG GGA ATG ACC-3') which includes a *Nco*I restriction enzyme site which also coincides with the start ATG codon of the sequence, and 3'-primer TG-84 (5'-AAG TCG CGA GCC GCG TTT GCC AC-3') which includes a site for the restriction enzyme *Nru*I and encodes for amino acids corresponding to position 64 of the PBP 1B sequence.

Step 1: The PCR amplified fragment following restriction with the enzymes *Nco*I and *Nru*I was cloned into the *Nco*I - *Nru*I sites of the cloning vector pBR 329 (Covarrubias et al., 1982). Ligation, transformation and screening were carried out using standard protocols and the recombinant plasmid with the expected structure labelled pARC0547 (Fig. 8) was obtained.

Another DNA fragment of approximately 1.2 kb was amplified by PCR using primer sequences corresponding to amino acid 87 to 480. This DNA fragment encodes the C-terminal half of the TG domain of PBP 1B. The primers used were 5'-primer TG-79 (5'-CGG ATA TCG ATC AAA AAA TTC GTA GCC G-3') which included the nucleotide sequence for the cleavage site for the restriction enzyme *Eco*RV, and 3'-primer TG-80 (5'-GCG GAT CCT TAG TCG ACG ACC ACA ATC GCA G-3') which included the sequence for *Bam*HI cleavage.

Step 2: The PCR amplification of this fragment was done using the *pon*B gene on pBS96 (Broome-Smith et al., 1985) DNA as template. The amplified fragment was cloned into the *Eco*RV - *Bam*HI sites of pBR 329 (Covarrubias et al., 1982) using standard protocols. The recombinant plasmid obtained was labelled pARC0534 (Fig. 8).

Step 3: The 200 bp *Nco*I - *Nru*I fragment cloned in pARC0547 was excised as a *Nco*I - *Nru*I fragment and cloned into *Nco*I - *Eco*RV cleaved pARC0534 to obtain pARC0551 (Fig. 8).

The mutant *pon*B gene on pARC0551 has DNA sequences coding for the N-terminal 64 amino acids of PBP 1B fused to the nucleotide sequences encoding the amino acids 88 to 480. A 1.3 kb *Pst*I - *Bam*HI DNA fragment of pBS96 was then ligated to *Pst*I - *Bam*HI cleaved pARC0551 and the ligation mixture transformed into *E.coli* DH5α using standard procedures. Individual transformants were then screened and colonies harbouring recombinant plasmid with the expected structure identified. The plasmid was labelled pARC0552. A *Nco*I - *Bam*HI fragment from pARC0552 encompassing the entire mutant *pon*B gene was then excised and ligated to the T7 expression vector pARC038 to obtain pARC0559 (NCIMB 40667; Fig. 9).

The 3'-end of the cloned fragment of Step 1 has the nucleotide sequence TCG (partial *Nru*I site sequence) while the 5'-end of the fragment cloned in Step 2 has the sequence ATC (partial *Eco*RV cleavage sequence). The junction nucleotide sequence which is the outcome of the fusion of TCG and ATC results in the introduction of the codons for serine and isoleucine. Thus the mutant *pon*B gene encodes a PBP 1B with the amino acid sequence 1 to 64 corresponding to the wild type PBP 1B fused to the sequence 87 to 844. The two stretches are joined by the amino acids serine and isoleucine.

The nucleotide sequence of the mutant *pon*B gene is shown as SEQ ID NO: 3 and the derived amino acid as SEQ ID NO: 4.

2.2. Expression of soluble PBP 1B

The plasmid DNA of pARC0559 was transformed into the T7 expression host *E.coli* BL 26 (DE3) and the restriction map profile of the transformed plasmid confirmed using standard procedures. *E.coli* BL26 (DE3)/pARC0559 were grown at 22°C and induced with 0.01 mM IPTG and the cells allowed to grow for 6 hours. Cells were then harvested and

broken by passage through a french press. The lysate was centrifuged at 10,000 rpm for 10 minutes and the supernatant obtained was centrifuged at 200,000 x g for 45 minutes in a Beckman ultracentrifuge.

2.3. Characterization of the expressed soluble PBP 1B

The obtained supernatant, i.e. the cytosolic / soluble fraction, was tested for the presence of the mutant PBP 1B using [^{125}I]ampicillin as the radio-ligand. The [^{125}I]ampicillin was prepared as described by Rojo et al. (1984) for the preparation of [^{125}I]cephradine. The mutant PBP 1B was detected in the soluble fraction and bound radioactive ampicillin.

Soluble PBP 1B could also be purified using Ampicillin - Affigel beads by a procedure analogous to the one described in Section 1.4. The protein profile of the different fractions seen by Coomassie Blue staining and the binding of [^{125}I]ampicillin of the enriched PBP 1B fraction is shown in Fig. 10.

The purified protein was enzymatically active in the peptidoglycan transglycosylase assay (Heijenoort et al., 1992) and bound penicillin with an affinity comparable to that of the membrane bound native PBP 1B.

EXAMPLE 3

3.1. Construction of gene encoding soluble form of *Streptococcus pneumoniae* PBP 1A

The molecular architecture of the *S.pneumoniae* PBP 1A is predicted to be similar to that of *E.coli* PBP 1A and PBP 1B protein in the fact that the protein is anchored to the membrane via a N-terminal membrane anchoring sequence. The nucleotide sequence of the gene encoding native membrane bound *S.pneumoniae* PBP 1A and its derived amino acid

sequence are described in Martin et al., (1992). The hydropathicity profile of the N-terminal 100 amino acids as derived by the Kyte and Doolittle plot is shown in Fig. 11. A stretch of 38 amino acids contributed significantly to the hydrophobicity of this region and was assumed to be the membrane interacting domain. A mutant gene of *S.pneumoniae* PBP 1A was constructed by deleting the nucleotide sequence coding for the N-terminal 38 amino acids of *S.pneumoniae* PBP 1A.

Using standard PCR protocols, sequences encoding the wild type *S.pneumoniae* PBP 1A gene was amplified as a 2.5 kb DNA fragment from the chromosome of *S.pneumoniae* strain PM1 (obtained from S.A. Lacks, Biology Department, Brookhaven National Laboratory, Upton, New York, USA) (Lacks, 1968) using the primers designed based on the sequence reported by Martin et al. (1992) and the amplified fragment cloned into the pneumococcal vector pLS 101 (Balganesh and Lacks, 1984).

The mutant gene encoding a soluble form of *S.pneumoniae* PBP 1A was constructed by using of plasmid DNA harbouring the wild type gene as template and amplifying a 2.3 kb DNA fragment by using PCR following standard procedures. The sequence of the primers used were 5'-primer TG-24 (5'-TAC GTT ACC ATG GCT CCT AGC CTA TCC-3') and 3'-primer TG-25 (5'-GAC AGG ATC CTG AGA AGA TGT CTT CTC A-3').

The 5'-primer TG-24 includes the sequence for the restriction enzyme *Nco*I while the 3'-primer TG-25 includes the site for the restriction enzyme *Bam*HI. The *Nco*I and *Bam*HI digested PCR amplified DNA fragment was ligated to *Nco*I - *Bam*HI cleaved pARC039. The plasmid pARC039 is a derivative of pET 8c (Studier et al., 1990) in which the gene coding for the β-lactamase has been replaced by a kanamycin resistance cartridge.

Following ligation and screening using standard protocols, the structure of the recombinant plasmid was confirmed by detailed restriction mapping

and transformed into the T7 expression host *E.coli* BL 21 (DE3) (Studier et al., 1990). The recombinant plasmid was labelled pARC0512 (NCIMB 40665) and is schematically represented in Fig. 12.

The nucleotide sequence of the mutant *S.pneumoniae* PBP 1A gene is shown as SEQ ID NO: 5 and the derived amino acid sequence is shown as SEQ ID NO: 6.

3.2. Expression and characterization of soluble form of *Streptococcus pneumoniae* PBP 1A

The gene coding for soluble *S.pneumoniae* PBP 1A was expressed by a procedure analogous to the one described in Section 1.2.. The cytosolic fraction of *E.coli* BL 21 (DE3)/pARC0512 was isolated and tested for the presence of the soluble form of the *S.pneumoniae* PBP 1Adel38. The radioactive ligand used for the binding studies was [^{3}H]benzyl penicillin (Amersham) which was prepared as described earlier. Approximately 50% of the expressed protein from the mutant gene was found to be in the soluble fraction and bound [^{125}I]penicillin (Rojo et al., 1984) or [^{3}H]penicillin (Amersham) when the culture was grown and induced at 22°C with 0.01 mM IPTG. The growth and induction conditions were critical for the efficient recovery of the soluble protein, as growth at higher temperatures or induction with higher concentrations of IPTG resulted in the majority of the protein becoming inactive and forming inclusion bodies. Optimum levels of soluble active protein was found following induction for 6-8 h. (Fig. 13).

The soluble *S.pneumoniae* PBP 1Adel38 protein could also be efficiently purified essentially following the protocol used for the purification of the soluble *E.coli* PBP 1B protein.

The efficiency of penicillin binding of the soluble PBP 1Adel38 was comparable to that of the native membrane bound *S.pneumoniae* PBP 1A.

EXAMPLE 4

4.1. Transglycosylase deficient *E.coli* PBP 1B

The conserved amino acids within Region 2 (Fig. 14) were chosen for site-directed mutagenesis. Within this stretch of 10 amino acids three different mutations were constructed:

(a) the glutamines at position 270 and 271 of the PBP 1B sequence were changed to alanines;

(b) the glutamines at position 270 and 271 of the PBP 1B sequence were changed to leucines; and

(c) a deletion of the nucleotide sequence encoding amino acids from position 264 to 271.

Mutants of the *pon*B gene were constructed essentially following the procedure of Kunkel et al. (1985). A 1.5 kb *Eco*RI - *Sal*I fragment of the *pon*B gene of the plasmid pBS96 was excised and cloned into *Eco*RI - *Sal*I cleaved M13mp19 following standard protocols.

(a) The primer used for mutating the nucleotide sequence coding for glutamine residues 270 and 271 into a sequence coding for alanine residues was TG-21:

```
5'-ACG CTG ACG GCC GCT CTG GTG AAA-3'
    T   L   T   A   A   L   V   K
```

(b) The primer used for mutating the sequence coding for the glutamine residues 270 and 271 into leucine residues was TG-23:

```
5'-ACG CTG ACG CTA TTG CTG GTG AAA-3'
```

T L T L L L V K

(c) The primer used for creating a deletion of the nucleotides encoding amino acids at position 264 to 271, all of which are within the conserved Region 2, was TG-22:

```
5'-CGC ACG GTA CAG CTG GTG AAA AAC-3'
    R   T   V   Q   L   V   K
    260 261 262 263 272 273 274  (amino acid no.)
```

Following mutagenesis, the nucleotide sequence of the mutagenized *Eco*RI - *Sal*I fragment was determined following the protocol of Sanger et al. (1977). The sequencing confirmed the nucleotide changes and also ruled out any extraneous changes. This mutated 1.5 kb DNA fragment was ligated back to *Eco*RI - *Sal*I cleaved pBS96 and the ligated DNA transformed in to *E.coli* DH5α cells following standard protocols. Kanamycin resistant transformants were analyzed for their plasmid profiles and the plasmid with the TG-21 mutation (a) was labelled pARC0438 (NCIMB 40661). The mutant protein is referred to as PBP 1B QQ-AA (SEQ ID NO: 7).

The plasmid with the mutation (b) introduced by TG-23 was labelled pARC0468 (NCIMB 40662). The mutant protein is referred to as PBP 1B QQ-LL (SEQ ID NO: 8)

The plasmid with the deletion (c) obtained using TG-22 was labelled pARC0469 (NCIMB 40663). The mutant protein is referred to as PBP 1Bdel8 (SEQ ID NO: 9).

The four plasmid DNAs of pBS96, pARC0438, pARC0468 and pARC0469 were individually transformed into *E.coli ponB*:spc^r cells (Broome-Smith et al., 1985) in which a deleted *ponB* gene had been marked with spectinomycin resistance marker.

E.coli ponB:spc^r cells having the individual plasmids pBS96, pARC0438 or pARC0469 were grown and membrane preparations made following the procedure described by Spratt (1977) and the profile of the penicillin binding proteins analyzed on a 8% SDS-PAGE following labelling with radioactive penicillin. The mutant proteins were initially analyzed for *in vivo* stability and localization into the membrane using anti-PBP 1B sera raised against purified membrane bound native PBP 1B (Fig. 15).

The mutant proteins were found to be localized to the membrane and no degraded protein fragments reacting with the antibody could be detected indicating no gross instability. In addition the mutant proteins bound penicillin with an affinity comparable to that of the wild type PBP 1B (Fig. 15).

After assaying for transglycosylase activity as described in Heijenoort et al. (1978), no activity could be detected in the membranes expressing the mutant proteins, while the membrane with the wild type PBP 1B showed transglycosylase activity. This defines the amino acids 263 to 271 as being critical for transglycosylase activity.

The ability of the mutant proteins to bind penicillin with an affinity comparable to that of the wild type suggests that the transpeptidase activity of the mutant proteins would also be comparable to that of the wild type. Knowing that the bifunctional protein PBP 1B expressed on a plasmid can in trans complement the deletions of both *ponA* and *ponB* (Yousif et al., 1985) the ability of the transglycosylase negative / transpeptidase positive proteins PBP 1B QQ-AA and PBP 1Bdel8 to complement the absence of chromosomally encoded PBP 1A and 1B was tested.

The wild type *ponB* and the mutant *ponB* genes were cloned into low copy vector pMAK 705 (Hamilton et al., 1989). The resulting plasmids were

designated pARC0462, (wild type *ponB*, Fig. 16), pARC0463, (*ponB*del8, Fig. 17) and pARC0470 (*ponB* QQ-AA, Fig. 18). The plasmids were individually transformed into *E.coli* del *ponA* (*E.coli* with a deletion of the *ponA* gene).

E.coli del *ponA* /pARC0462, *E.coli* del *ponA*/pARC0463 and *E.coli* del *ponA*/pARC0470 were used as recipients of the P1 phage for the transduction of the *ponB*:spc^r marker. The transduction was performed as described by Miller (1972) The phage P1 lysate was made on *E.coli* *ponB*:spc^r strain (Yousif et al., 1985). Following infection, the infected cells were plated on spectinomycin. Integration of the DNA fragment harbouring and *ponB*:spc^r transduced into any of the recipients results in the inactivation of the chromosomal *ponB* gene rendering the chromosome *ponA*$^-$ and *ponB*$^-$. This genotype being lethal for the cell, the *E.coli* spectinomycin resistant transductants can remain viable only if the plasmid encoded *ponB* or the *ponB* mutant can functionally complement in trans.

The following *E.coli* strains were subject to phage P1 transduction analysis of trans-complementation: (1) *E.coli* AMA 1004 which has chromosomally coded wild type *ponA* and *ponB*; (2) *E.coli* AMA 1004 which has a chromosomally inactivated *ponB* and is the host for the plasmid coded mutant *ponB* genes; (3) *E.coli* AMA 1004 host bearing the plasmid pARC0462 encoding the wild type *ponB* gene; (4) *E.coli* AMA 1004 host bearing the plasmid pARC0463 encoding PBP 1Bdel8; and (5) *E.coli* AMA 1004 host bearing the plasmid pARC0470 encoding PBP 1B QQ-AA.

Results (Number of Km^r transductants /-ml)

(1) *E.coli* AMA 1004	3.0×10^4
(2) *E.coli* AMA 1004, *pon*B:spc^r	< 1
(3) *E.coli* AMA 1004, *pon*B:spc^r (PBP 1B wt)	1.1×10^4
(4) *E.coli* AMA 1004, *pon*B:spc^r (PBP 1Bdel8)	< 1
(5) *E.coli* AMA 1004, *pon*B:spc^r (PBP 1B QQ-AA)	< 1

A comparable number of transductants were obtained for an internal marker : trp transduction using the same P1 phage lysate.

The above results show that viable transductants could be obtained only with wild type PBP 1B, indicating that the TG^- TP^+ product encoded by *pon*B QQ-AA or *pon*Bdel8 could not complement the loss of chromosomally encoded PBP 1A and 1B. However, as these mutant proteins bind penicillin and thus can be assumed to have transpeptidase activity, the inability to complement must be the absence of the transglycosylase enzymic activity. These results confirm the essential nature of the transglycosylase activity of PBP 1A or 1B for the viability of the *E.coli* cell.

The mutants described define the Region 2 to be involved in the transglycosylase activity of the protein. As this stretch of amino acids is conserved within the four high molecular weight penicillin binding proteins namely *E.coli* PBP 1A, 1B and *S.pneumoniae* 1A and the 94 kDa protein of *H.influenzae* (Fig. 14) it is reasonable to assume similar catalytic or structural involvement of this region in all the transglycosylase enzymes utilizing substrates similar to that used by PBP 1A and 1B of *E.coli*.

4.2. Transglycosylase deficient *E.coli* PBP 1A

The conserved Region 2 was chosen for site-directed mutagenesis and the nucleotide sequence coding for glutamine at positions 123 and 124 of *E.coli*

PBP 1A was changed to a sequence coding for alanine by PCR mutagenesis as follows. The 5' half of the *pon*A gene was amplified as 2 fragments, the 5'-fragment corresponding to amino acid 1 to 123 (fragment A) and the 3'-fragment corresponding to amino acid 124 to 434 (fragment B).

The sequence of the 5'-primer used for the amplification of fragment A was TG-93 (5'- GCG CGG ACC ATG GTG AAG TTC GTA AAG TAT-3') while the 3'-primer used for the amplification of fragment A was TG-106 (5'-CAG TGC TGC AGT AAT GGT ACT TGC CCC TTG-3').

The 3'-primer for fragment A amplification included the sequence for the restriction enzyme *Pst*I which allowed the conversion of the sequence encoding the glutamine residues in position 123 and 124 into a nucleotide sequence coding for alanine residues.

Fragment B was amplified with the 5'-primer TG-107 (5'-ATT ACT GCA GCA CTG GCG AGA AAC TTC TTC-3') and the 3'-primer TG-108 (5'-TCG CGA GAT ATC TGG CGG ATT GAT CGA CAC-3').

The 5'-primer for amplifying fragment B included the sequence for the restriction enzyme *Pst*I overlapping the sequence with that of 3'-primer for amplifying fragment A. Ligation of the 3'-end of fragment A to the 5'-end of fragment B recreated the site for *Pst*I and resulted in the change of the nucleotide sequence encoding glutamine 123 and 124 into alanine 123 and 124. The amplified fragments A and B were individually cloned into pBR 329, and corresponding clones pARC0565 and pARC0566 were obtained.

Fragment A and B obtained from pARC0565 and pARC0566 were ligated to obtain pARC0567. The *pon*A sequences were completed by introducing an *Xho*I - *Bam*HI fragment of pARC0489 (which is identical to pARC0558 (Fig. 3) except for having additional *Lac*I and Lac operator sequences) into pARC0567 to obtain pARC0568. The *Mlu*I - *Bgl*II fragment of pARC0568

which included the Q_{123} - Q_{124} to A_{123} - A_{124} mutated region was then used to replace the otherwise identical *Mlu*I - *Bgl*II fragment of pBS98 to obtain the plasmid pARC0571 (Fig. 19; NCIMB 40668). The mutant protein was labelled PBP 1A QQ-AA (SEQ ID NO: 10).

Expression studies on the mutant indicated that the mutant protein was localised to the membrane (as detected by anti PBP 1A antibodies) and bound penicillin with an affinity comparable to that of the native PBP 1A (Fig. 20).

An *in vivo* complementation assay, similar to that described in the previous section, was performed by checking the ability of mutant PBP 1A protein to complement in trans. The *in vivo* complementation was performed using phage P1 transduction and transducing *pon*B:spc^r into the host *E.coli* (recipient) del *pon*A harbouring the plasmid encoding the mutant protein PBP 1A QQ-AA.

In order to carry out the complementation analysis the wild type *pon*A gene was cloned into the low copy vector pMAK 705 (Hamilton et al, 1989) to obtain pARC0583 and the mutant *pon*A gene encoding PBP 1A QQ-AA cloned into pMAK 705 to obtain pARC0582.

The following *E.coli* strains were subject to phage P1 transduction analysis of trans-complementation: (1) *E.coli* AMA 1004 which has chromosomally coded *pon*A and *pon*B; (2) *E.coli* AMA 1004 *pon*A which has a chromosomally inactivated *pon*A and is the host for the plasmid coded mutant *pon*A genes; (3) host bearing the plasmid pARC0583 encoding the wild type *pon*A gene; (4) host bearing the plasmid pARC0582 encoding PBP 1A QQ-AA.

Results (Number of Spc^r transductants / ml)

(1) *E.coli* AMA 1004	2.1×10^3
(2) *E.coli* AMA 1004, *ponA*	< 1
(3) *E.coli* AMA 1004, *ponA* (PBP 1A wt)	1.64×10^3
(4) *E.coli* AMA 1004, *ponA* (PBP 1A QQ-AA)	< 1

A comparable number of transductants were obtained for internal marker : trp transduction using the same P1 phage lysate.

As shown above, no viable transductants could be obtained with *E.coli* del *ponA* / pARC0582 as recipient indicating that the mutant PBP 1A QQ-AA could not complement the absence of chromosomally encoded PBP 1A/1B. This indicates that the Q_{123} and Q_{124} of region 2 of PBP 1A also affects transglycosylase activity of the protein as the loss of the complementing function must be a reflection of the loss of transglycosylase activity. The transpeptidase activity of the protein is unaffected as tested by its affinity to bind penicillin.

These results argue in favour of the region 2 as a critical stretch of amino acids involved in the transglycosylase enzymic function and may be the explanation for the strong evolutionary conservation of this stretch of amino acids.

EXAMPLE 5

5.1. Truncated *E.coli* PBP 1B

A mutant gene encoding the truncated PBP 1B consisting of the N-terminal 553 amino acids was constructed by PCR amplification using the 5'-primer TG-77 (5'-GAA AAA CCA TGG CCG GGA ATG ACC-3') and the 3'-primer TG-116 (5'- ATG GGA TCC TTA ATC ATT CTG CGG TGA-3').

The 5' end of the primer corresponded to the amino acid 553 in the wild type followed by the stop codon and a site for the restriction enzyme *Bam*HI. A fragment of 1.7 kb was amplified using pBS96 DNA as template. The PCR amplified fragment was cut with *Pst*I and *Bam*HI and cloned into *Pst*I-*Bam*HI restricted pARC0555 (pARC0555 has the full length *pon*B gene cloned as *Nco*I-*Bam*HI fragment into the expression vector pET11d. The *Nco*I site includes the initiation codon ATG) to obtain pARC0592 (NCIMB 40669; Fig. 21) The expressed protein (SEQ ID NO: 11) was shown to have transglycosylase activity, thus confirming the functional independence of this domain.

The soluble truncated PBP 1B, i.e. PBP 1B with N-terminal 553 amino acids but lacking the membrane anchoring hydrophobic domain from 65-87, was constructed by replacing the *Pst*I-*Bam*HI fragment of pARC0559 (Fig. 9) with the *Pst*I-*Bam*HI fragment of pARC0592 to obtain pARC0593 (NCIMB 40670; Fig. 22). The mutant *pon*B gene encodes the soluble form of PBP 1B and the expressed protein (SEQ ID NO: 12) was found to have transglycosylase activity.

5.2. Minimum substrate binding domain of truncated *E.coli* PBP 1B

Detailed computer analysis of the anatomy of the presumptive TG domain (aa 1-553) of PBP 1B indicated that aa 210-368 were probably sufficient for the binding of the lipid linked substrate and the transglycosylase reaction. This stretch of amino acids includes the 3 conserved domains Region I, II and III. The mutant gene encoding the truncated protein stretch 210-368 was constructed as follows.

A fragment of approx size 480 bp was amplified from pBS96 as substrate with the 5'-primer having the sequence TG-154 (5'-CAA TCC ATG GGT GAG CAG CGT CTG TTT G-3') were the initiation ATG codon is

immediately followed by the sequence encoding the 210th amino acid of PBP 1B.

The 3'-primer corresponded to the sequence TG-155 (5'-T CCA GAA TTC CAG TTT TGG GTT ACG-3') were the sequence encoded the amino acid 368 of PBP 1B followed by the nucleotide sequence that provides the restriction site for *Eco*RI, enabling fusion to sequences encoding an enterokinase site and a histidine stretch, which allows rapid purification of the protein on an Ni affinity column (cf. section 6.2 below).

A *Nco*I-*Eco*RI fragment was cloned into the plasmid pARC0400 that was restricted with *Nco*I-*Eco*RI to obtain the recombinant plasmid pARC0392 (NCIMB 40659; Fig. 23). The recombinant plasmid was transformed into *E.coli* BL26 (DE3) and a protein of approximately 17 kDa was detected largely in the soluble fraction after induction with IPTG.

Along similar lines the minimum substrate binding region of PBP 1A could be predicted to involve the stretch 62-220 in the wild type protein. Production of this protein as a fusion with a histidine stretch allows high efficiency affinity purification of the expressed product using the Ni^{2+} column. That the results will be similar to that obtained with truncated PBP 1B can be anticipated.

EXAMPLE 6

6.1. N-terminal fusion of soluble *E.coli* PBP 1A to glutathione-S-transferase

Fusion of the *pon*Adel23 gene at its 5'-end in frame to sequences coding for glutathione-S-transferase was made as described in the following section.

The vector chosen for the fusion gene construction was pGEX-3X obtained from Pharmacia Biochemicals. In order to fuse the 5'-initiation ATG of

*pon*Adel23 in frame with the gene encoding glutathione-S-transferase a *Bam*HI site was introduced using a PCR primer whose sequence included the sequence for the restriction enzyme *Eco*RI. The 5'-primer used was TG-115:

5'-TCG AGG ATC CCC ATG GGC CTA TAC CGC TAC ATC G-3'
======== ========
*Eco*RI *Bam*HI

The 3'-primer used was TG-106, described in Section 4.2. The PCR amplified DNA Fragment A was digested with *Bam*HI and *Pst*I and cloned into the *Bam*HI - *Pst*I sites of the standard cloning vector pUC8 to obtain pARC0496. This Fragment A includes the N-terminal 102 amino acids of the PBP 1Adel23 protein. A *Bam*HI - *Mlu*I (site present within the fragment A) 270 bp fragment obtained from Fragment A, a 2.2 kb *Mlu*I - *Eco*RI fragment which includes the rest of the portion of the *pon*A gene obtained from pARC0490 (pARC0490 has the wild type *pon*A gene cloned into the *Xba*I - *Bam*HI sites of the low copy vector pWKS29 (Fu Wang et al., 1991) facilitating the 3'-end of the *pon*A del 23 gene to be excised as an *Eco*RI fragment) and a *Eco*RI - *Bam*HI cleaved pGEX-3X were ligated together and transformed into competent *E.coli* cells. Individual transformants were screened for recombinant plasmid and the plasmid with the expected structure was designated pARC0499 (NCIMB 40664; Fig. 24). The encoded fusion product on pARC0499 has the glutathione-S-transferase sequences at its C-terminus linked to PBP 1Adel23 sequences via a Factor Xa cleavage recognition sequence.

Following induction with 1 mM IPTG, a fusion protein of expected size was found to be induced. The protein bound penicillin and was active in the transglycosylase assay. Following cell lysis by passing the suspension through a French press, the cell free supernatant fraction was prepared as detailed in Section 1.4. for the purification of PBP 1Adel23. The supernatant fraction was passed through a Glutathione Sepharose® matrix

(Pharmacia Biochemicals) and the bound GST-PBP 1Adel123 was eluted with glutathione. The eluted protein was found to be 80% homogeneous. Free glutathione was removed by dialysis and the GST-PBP 1Adel 23 was cleaved with factor Xa.

PBP 1Adel23 thus purified was found to be active in both penicillin binding and the transglycosylase reactions.

6.2. C-terminal fusion of soluble *E.coli* PBP 1A to histidine stretch

Fusion of the *pon*Adel23 gene at its 3'-end in frame to sequences encoding a stretch of 6 histidines was made as described below.

In the first step the *pon*Adel23 gene was amplified using pBS98 DNA as template using the 5'-primer TG-115 (5'-TCG AGG ATC CCC ATG GGC CTA TAC CGC TAC ATC G-3') and the 3'-primer TTG-121 (5'-GTT AGA ATT CGA ACA ATT CCT GTG-3').

The 3'-primer introduced an *Eco*RI site at the 3' end of the *pon*Adel23 gene while eliminating the translation stop codon. The PCR amplified modified *pon*Adel23 gene fragment was digested with *Pst*I and *Eco*RI to release a 930 bp 5'-end fragment and ligated to *Pst*I-*Eco*RI digested pBR 329 to obtain the recombinant plasmid pARC0467.

In the next step, a double stranded synthetic oligonucleotide with sequences encoding the six histidines and the DNA sequence coding for amino acids recognised as the enterokinase cleavage site was synthesised and ligated to the newly created *Eco*RI site at the 3'-end of the *pon*Adel23 gene on pARC0467. The synthetic oligonucleotides used were TG-122:

```
    EcoRI
    ------
5'-AA TTC GAC GAC GAC GAC AAG CAC CAC CAC CAC CAC CAC TGA TAA G-3'
          ----------------------==========================
5                ENTEROKINASE              HISTIDINES
```

and TG 123 (5'-GAT CCT TAT CAG TGG TGG TGG TGG TGG TGC TTG TCG TCG TCG TCG-3').

The plasmid pARC0467 was linearised with *Eco*RI and the synthetic double stranded oligonucleotide ligated. Following ligation a *Pst*I - *Bam*HI (Fragment A) was released from the ligation mixture and cloned into the *Pst*I - *Bam*HI sites of pARC0558 (Fig. 3), to obtain pARC0400 (NCIMB 40660; Fig. 25). The mutant *pon*Adel23 fusion gene thus encoded a protein with the PBP 1Adel23 sequence fused to the amino acid sequence Asp-Asp-Asp-Asp-Lys fused to His-His-His-His-His-His at its C-terminus. The Asp-Asp-Asp-Asp-Lys sequence is recognised by the protease enterokinase and cleaves following the lysine residue. The six histidine residues confer on the protein the ability to bind to the metal nickel.

The recombinant plasmid pARC0400 was transformed in *E.coli* BL26(DE3) cells and induced under culture and temperature conditions identical to those used for the purification of PBP 1Adel23. The cells were lysed by passing through a French press. The lysate was centrifuged at 10,000 rpm for 10 min. The supernatant obtained after low speed centrifugation was then spun at 200,000 x g for 45 min and the supernatant obtained represented the cytosolic fraction. This fraction contained the protein encoded by the fusion gene and the recombinant fusion protein was labelled PBP 1Adel23EH. This protein PBP 1Adel23EH bound [^{125}I]cephradine and was also active in transglycosylase assay. The soluble fraction was passed through a Ni affinity column and bound protein eluted in batches with increasing concentrations of imidazole essentially following the procedure described in "The Qia Expressionist" obtained from QIAGEN Inc. 9259 Eton Avenue, Chateworth, CA 91311 USA. The majority of PBP

1Adel23EH eluted with 250 mM imidazole and was approximately 85% homogenous. It was the only cephradine binding protein eluted from the column. Thus the ability of fusion protein to bind to the Ni column can be easily exploited both for efficient purification and immobilisation of the active protein.

EXAMPLE 7

7.1. Use of cell extracts for enzyme assays and in screening

The crude cell extract made according to Example 6 can be analyzed for the ability to bind penicillin by reacting with [^{3}H]ampicillin prepared according to Hackenbeck (1983). To adapt the procedure to large-scale screening, a 96 well microtitre plate is used to contain the reactions and the assay is performed using a Beckman Biomek robot. Crude cell extract is mixed with [^{3}H]ampicillin for 15 min at 37°C. The proteins in the reactionare are precipitated with TCA and collected on a glass filter, unbound ampicillin is washed off and filters counted in a scintillation counter. Alternatively, autoradiography can be used to assay the degree of binding of ampicillin.

Based on the above method, a competitive assay can be used to assess the ability of test compounds to bind to the transpeptidase site of a PBP variant. In this assay, the test compound is exposed to the crude cell extract for 15 min prior to the addition of ampicillin. A positive result is indicated by a reduction in the amount of radioactivity present on the glass filter.

7.2. Use of soluble immobilised protein in screening

Protein containing a histidine peptide which has been purified as described can be used for screening for compounds which inhibit transpeptidase

activity or transglycosylase activity. The purified full length or truncated protein is immobilised onto agarose gel to which Ni(II) has been coupled. Aliquots of the beads containing immobilised protein are then transferred to the wells of a microtitre plate, test compounds are added to the plate and incubated before unbound test substance is washed free. Compounds which bind to the transpeptidase site of the bifunctional protein can be detected by adding [^{3}H]ampicillin to the reaction vessel and continuing essentially as described above. Alternatively monoclonal antibodies known to bind to the transpeptidase region can be used. Compounds which bind to the transglycosylase site can be assessed in a competitive assay by the use of monoclonal antibodies which bind to the transglycosylase region of the protein.

EXAMPLE 8

8.1. Production of monoclonal antibodies to PBP 1A

The protocol for the production of monoclonal antibodies (mAbs) was essentially that described in "Antibodies - a laboratory manual" (ed. Harlow David Lane, Cold Spring Harbor, USA). Purified membrane bound PBP 1A was used as the immunogen. Balb-C mice, 6-8 weeks old were immunised with 50 µg of purified native PBP 1A in Freunds Complete Adjuvant. A booster injection of 20 µg PBP 1A in incomplete Freunds adjuvant was given intraperitonially. Two weeks later the presence of serum antibodies was checked by ELISA using PBP 1A as the coated antigen. Mice with circulating antibodies were immunised intraperitonially daily for 4 days with 20 µg of PBP 1A in saline and the mice sacrificed for isolating splenocytes for generating fusions.

The myeloma cell line used in fusion experiments was Sp 2/0-Ag 14 and these cells were fused with splenocytes from immunised mice at an ratio of 10 : 1. Fusion was carried out using standard protocols and antibody

production from the clones was monitored by ELISA against PBP 1A when the cells were > 90% confluent.

72 high producing clones were expanded to 24 well plates and the secreted antibody characterised using the following screens: (1) ELISA against membrane bound form of PBP 1A; (2) ELISA against soluble form of PBP 1Adel 23; (3) Dot blot analysis against membrane bound PBP 1A to eliminate monoclonals reacting with the detergent solubilised purified PBP 1A protein only due to changes in the configuration during purification; and (4) ELISA against membrane bound form of PBP 1B.

Based on these screens, a panel of 5 secreting clones were selected and subcloned twice to ensure monoclonality. Ascites with these hybridoma clones were raised following standard procedures and IgG was purified from these ascites fluids, using Protein G-Sepharose® affinity chromatography as recommended by the manufacturers of Protein G-Sepharose® (Pharmacia Biochemicals).

These purified antibodies react specifically with PBP 1A in both the membrane bound and the soluble forms in ELISA, Dot blots and in Western blotting. Clones were obtained by a cloning procedure employing 3 cells / well. To ensure the monoclonality these clones were subcloned into 96 well microtitre plates by limiting dilution at 1 cell / well. The wells receiving one cell were carefully confirmed under the microscope and allowed to grow with macrophage feeder layers so as to obtain progeny from a single hybrid cell. Following sub-cloning the secretion of mAbs to PBP 1A was again assayed in ELISA using full length PBP 1A. Finally two clones from each parent hybridoma were selected and one of them was expanded as ascites in pristine primed Balb/c mice. All the five clones adapted to grow in peritoneal cavities and produced ascitic mAbs.

The ascitic mAbs were titrated against purified PBP 1A in ELISA. All the ascitic mAbs had a titre of > 5 x 10^5 in ELISA and recognised full length protein in western immunoblots. The ascitic mAbs were purified by protein-G affinity columns.

The immunoglobulin isotype of mAbs was determined by mouse Ig - isotype by ELISA using a kit obtained from Sigma chemicals USA. Four of the monoclonals belonged to IgG1 and one belonged to IgG2a immunoglobulin isotype.

Further characterization of mAbs was done by using full length membrane bound PBP 1A/1B in western blots. In addition the transglycosylase (TG) and transpeptidase (TP) domain specificity of mAbs was determined by using various truncated forms of the membrane-bound N-terminal of PBP 1A, N-terminal of PBP 1B and C-terminal of PBP 1B in Western immunoblots. Various full length and truncated membrane bound PBPs were expressed and the prepared membrane fractions were resolved on a SDS-PAGE. The proteins were transferred onto nitrocellulose membranes and subjected to western blot analysis using polyclonal *E.coli* PBP 1A antibodies and monoclonal antibodies.

Assessment of the penicillin binding inhibitory potential of the mAbs was determined essentially following the protocol described by den Blaauwen et al. (1990). The protein-G affinity purified mAbs was preincubated with PBP 1A followed by addition of [^{3}H]benzyl penicillin or [^{125}I]cephradine. Two of the mAbs competitively inhibited binding of the radiolabelled penicillin to PBP 1A.

Monoclonal antibodies specific for the TG domain of PBP 1A have been obtained by screening the secreted antibody of the original hybridoma clones to react with the protein representing the N-terminal 434 amino acids of PBP 1A in western blots. Antibody from clone TG-2 reacted with

the N-terminal truncated 434 amino acid analogue of PBP 1A but also inhibited (>80% inhibition) the transglycosylase activity of PBP 1A. This indicates that the antibody recognises sequences in the protein which are involved in (a) binding of the substrate; (b) catalysing the enzymic action; or (c) altering conformation of the protein allosterically. In either of the three possibilities, identification of compounds competing for the binding of TG-2 to PBP 1A would represent molecules interacting with identical sequences on PBP 1A. Thus the competitive binding assay could be used as a screening assay for the identification of the TG inhibitory compound.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1

Hydropathicity profile of *E.coli* PBP 1A. The figure shows in expanded form the hydropathicity pattern of the N-terminal 55 amino acids of PBP 1A.

Figure 2

Schematic representation of the T7 translation fusion expression vector pARC038.

—— vector sequences

═══ gene conferring kanamycin resistance Km^r, gene encoding the lactose repressor (lac I_q), origin of replication (ori), T7 lac operator promoter, T7 phage terminator.

The direction of transcription of the different genes are shown by arrows. Relevant restriction enzyme sites are shown. Numbers next to the restriction site represent the nucleotide position taking the nucleotide at the upper twelve o'clock-position as zero.

Figure 3

Schematic representation of the vector pARC0558 encoding soluble PBP 1Adel 23 of *E.coli*.

—— vector sequences

═══ mutant gene encoding PBP 1Adel23, kanamycin resistance Km^r, lactose repressor (lac Iq) and the origin of replication ori.

Figure 4

Expression of soluble PBP 1Adel23. Panel A represents the autoradiogram of the [^{125}I]cephradine binding profile of the uninduced and induced cultures of *E.coli* BL 26 (DE3) harbouring pARC0558. Panel B represents the Coomassie Brilliant Blue staining protein profile of the same uninduced and induced cells. Lane (1): uninduced cytosol fraction; (2): uninduced membrane fraction; (3): induced cytosol fraction; (4): induced membrane fraction; (M): molecular weight markers.

Figure 5

SDS-PAGE pattern of purified PBP 1Adel23. Panel A: Coomassie blue staining. Panel B: [^{125}I]cephradine binding protein profile. Lanes (1): *E.coli* BL 26(DE3)/pARC0558 cytosolic fraction (200,000g supernatant); (2): 30% Ammonium sulphate supernatant fraction; (3): 30% Ammonium sulphate pellet fraction; (4): Cephradine affigel breakthrough fraction; (5): Molecular weight markers; (6-8): Cephradine affigel eluate.

Figure 6

Transglycosylase activity profile of wild type PBP 1A and mutant PBP 1Adel23 using purified proteins.

(▲—▲) represents activity of soluble PBP 1Adel23;

(●—●) represents activity of membrane bound PBP 1A solubilised with octyl-β-glucoside. X-axis represents the concentration of the proteins used in μg. Y-axis represents the quantities of peptidoglycan formed.

Figure 7

Hydropathicity profile of *E.coli* PBP 1B. The figure represents the expanded hydropathicity profile of the N-terminal 150 amino acids of *E.coli* PBP 1B.

Figure 8

Schematic representation of the cloning of the soluble transglycosylase domain of *E.coli* PBP 1B.

—— vector sequences

═══ sequences encoding *pon*B gene fragments and β-lactamase

The *Nco*I-*Nru*I fragment encoding the N-terminal 64 amino acids of PBP 1B was cloned into the *Nco*I-*Eco*RV sites of pARC0534 to obtain the plasmid pARC0551. This recombinant plasmid harbours the gene encoding amino acid 1 to 480 of PBP 1B with internal deletion of amino acid 65 to 87.

Figure 9

Schematic representation of pARC0559 encoding soluble PBP 1B.

—— vector sequences

═══ sequences of the mutant *pon*B gene encoding the soluble form of PBP 1B (solPBP 1B), lactose repressor (lac I_q), kanamycin resistance (Km^r) and the origin of replication (ori).

Arrows represent direction of transcription of the genes.

Figure 10

Purification of soluble PBP 1B. Panel A: SDS-PAGE, Coomassie blue staining of the different fractions. Panel B: [^{125}I]ampicillin binding profile of the same fractions. Lanes (1) and (2): Cytosolic fraction of *E.coli* BL 26(DE3)/pARC0559 induced cells; (3): Breakthrough fraction of Ampicillin-Affigel column; (4): Molecular weight markers; (5) and (6): Eluted fraction from the Ampicillin-Affigel column.

Figure 11

Hydropathicity profile of *S.pneumoniae* PBP 1A. The figure shows the expanded profile of the hydropathicity profile of the N-terminal 100 amino acids of *S.pneumoniae* PBP 1A.

Figure 12

Schematic representation of the plasmid pARC0512 encoding soluble form of *S.pneumoniae* PBP 1A.

—— represents vector sequences

═══ represents sequences of the gene encoding soluble PBP 1A of *S.pneumoniae* (sPBP 1A), kanamycin resistance Km^r and the origin of replication (ori).

Figure 13

Penicillin binding profile of soluble *S.pneumoniae* PBP 1A. Host: *E.coli* BL 21(DE3)/pARC0512. Panel A: Coomassie Blue staining. Panel B: *In vivo* labelling with [^{3}H]benzyl penicillin followed by SDS-PAGE. Lanes (1) and (2): Cytosolic fraction of cells induced at 22°C for 2 h and 20 h respectively; (3): Cytosolic fraction of cells induced at 30°C for 2 h; (4): Cytosolic fraction of cells induced at 37°C for 2h; (5): Molecular weight markers.

Figure 14

Amino acid alignment of conserved regions of the transglycosylase domain of high molecular weight penicillin binding proteins. The figure compares the conserved residues of the Regions 1, 2 and 3 among E.1A (*E.coli* PBP 1A), E.1B (*E.coli* PBP 1B), S.1A (*S.pneumoniae* PBP 1A), and H.inf (*Haemophilus influenzae* PBP 1A). (*) indicates identical amino acid residues.

Figure 15

Analysis of membrane protein of *E.coli* cells harbouring plasmids with genes encoding mutant PBP 1B. Panel A: [^{3}H]benzyl penicillin binding profile. Panel B: Western blotting with anti-PBP 1B sera. Lanes (1): Molecular weight markers; (2): Membrane fraction of *E.coli* JM 101/pBS96

cells; (3): Membrane fraction of *E.coli* 900521 *pon*B:Spc^r cells (This host lacks chromosomal encoded PBP 1B); (4): Membrane fraction of *E.coli* 900521 *pon*B:spc./pARC0438 cells; (5): Membrane fraction of *E.coli* 900521 *pon*B:spc/pARC0469; (6): Membrane fraction of *E.coli* 900521 *pon*B:spc/pARC0468.

Figure 16

Schematic representation of plasmid pARC0462 encoding wild type PBP 1B:

—— vector sequences

═══ sequences of the *pon*B gene, replication origin (ori), chloramphenicol acetyl transferase (cm^r) and portions of the lac Z multiple cloning site.

Figure 17

Schematic representation of plasmid pARC0463 encoding mutant *pon*B gene.

—— vector sequences

═══ sequences of mutant the *pon*B gene encoding PBP 1Bdel8 amino acids, replication origin (ori), chloramphenicol acetyl transferase (cm^r) and portions of the lac Z multiple cloning site.

Figure 18

Schematic representation of plasmid pARC0470 encoding mutant *pon*B gene.

—— vector sequences

═══ sequences of mutant the *pon*B gene encoding PBP 1B $Q_{271\text{-}272}$ - $A_{271\text{-}272}$, replication origin (ori), chloramphenicol acetyl transferase (cm^r) and portions of the lac Z multiple cloning site.

Figure 19

Schematic representation of pARC0571 harbouring mutant *pon*A gene.

—— vector sequences

═══ sequences of mutant *pon*A gene (PBP 1A QQ-AA), kanamycin resistance Km^r origin of replication (ori).

Figure 20

[^{125}I]Penicillin binding protein profile of wild type and mutant *E.coli* PBP 1A. Lane (1): *E.coli* AMA 1004 *pon*B:spc^r/pBS 98 (w.t. *pon*A); (2): *E.coli* BL21 (DE3) *pon*B:spc^r/pARC0570 (w.t. *pon*A); (3): *E.coli* AMA 1004 del *pon*A/pARC0571 (QQ-AA *pon*A); (4): *E.coli* AMA 1004 del *pon*A/pBS 98 (w.t. *pon*A); (5): Molecular weight markers.

Figure 21

Schematic representation of plasmid pARC0592.

—— vector sequences

═══ sequences of truncated *pon*B gene encoding for the N-terminal 553 amino acids of PBP 1B (hinge 1B), kanamycin resistance (Km^r) and origin of replication (ori)

Figure 22

Schematic representation of plasmid pARC0593.

—— vector sequences

═══ sequences of mutant truncated *pon*B gene encoding a soluble form of the truncated N-terminal 553 amino acids of PBP 1B (soluble hinge 1B), kanamycin resistance Km^r and origin of replication (ori).

Figure 23

Schematic representation of plasmid pARC0392.

—— vector sequences

═══ sequences of mutant gene encoding truncated fragment of PBP 1B protein, representing amino acids 210-368 sequences fused in frame at its 3'-end to sequences encoding a enterokinase site followed by a

stretch of 6 histidines, kanamycin resistance Km^r and origin of replication (ori).

Figure 24

Schematic representation of plasmid pARC0499.

—— vector sequences

═══ sequences of mutant *pon*Adel23 gene fused at its 5'-end in frame to sequences encoding Glutathione-S-transferase encoding sequences, β-lactamase amp^r and origin of replication (ori).

Figure 25

Schematic representation of plasmid pARC0400.

—— vector sequences

═══ sequences of mutant *pon*Adel23 sequences fused in frame at its 3'-end to sequences encoding a enterokinase site followed by a stretch of 6 histidines, kanamycin resistance Km^r and origin of replication (ori).

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(ii) TITLE OF INVENTION: Novel Polypeptides

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2487 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: DH5 alpha

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: PCR cloning
(B) CLONE: pARC 0558 Soluble PBP 1A del 23

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2487

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..2484

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATG GGC CTA TAC CGC TAC ATC GAG CCA CAA CTG CCG GAT GTG GCG ACA        48
Met Gly Leu Tyr Arg Tyr Ile Glu Pro Gln Leu Pro Asp Val Ala Thr
  1               5                  10                  15

TTA AAA GAT GTT CGC CTG CAA ATT CCG ATG CAG ATT TAC AGC GCC GAT        96
Leu Lys Asp Val Arg Leu Gln Ile Pro Met Gln Ile Tyr Ser Ala Asp
            20                  25                  30

GGC GAG CTG ATT GCT CAA TAC GGT GAG AAA CGT CGT ATT CCG GTT ACG       144
Gly Glu Leu Ile Ala Gln Tyr Gly Glu Lys Arg Arg Ile Pro Val Thr
        35                  40                  45
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TTG GAT CAA ATC CCA CCG GAG ATG GTG AAA GCC TTT ATC GCG ACA GAA      192
L u Asp Gln Ile Pro Pro Glu M t Val Lys Ala Phe Ile Ala Thr Glu
     50                  55                  60

GAC AGC CGC TTC TAC GAG CAT CAC GGC GTT GAC CCG GTG GGG ATC TTC      240
Asp Ser Arg Phe Tyr Glu His His Gly Val Asp Pro Val Gly Ile Phe
 65                  70                  75                  80

CGT GCA GCA AGC GTG GCG CTG TTC TCC GGT CAC GCG TCA CAA GGG GCA      288
Arg Ala Ala Ser Val Ala Leu Phe Ser Gly His Ala Ser Gln Gly Ala
                 85                  90                  95

AGT ACC ATT ACC CAG CAG CTG GCG AGA AAC TTC TTC CTC AGT CCA GAA      336
Ser Thr Ile Thr Gln Gln Leu Ala Arg Asn Phe Phe Leu Ser Pro Glu
            100                 105                 110

CGC ACG CTG ATG CGT AAG ATT AAG GAA GTC TTC CTC GCG ATT CGC ATT      384
Arg Thr Leu Met Arg Lys Ile Lys Glu Val Phe Leu Ala Ile Arg Ile
        115                 120                 125

GAA CAG CTG CTG ACG AAA GAC GAG ATC CTC GAG CTT TAT CTG AAC AAG      432
Glu Gln Leu Leu Thr Lys Asp Glu Ile Leu Glu Leu Tyr Leu Asn Lys
    130                 135                 140

ATT TAC CTT GGT TAC CGC GCC TAT GGT GTC GGT GCT GCG GCA CAA GTC      480
Ile Tyr Leu Gly Tyr Arg Ala Tyr Gly Val Gly Ala Ala Ala Gln Val
145                 150                 155                 160

TAT TTC GGA AAA ACG GTC GAC CAA CTG ACG CTG AAC GAA ATG GCG GTG      528
Tyr Phe Gly Lys Thr Val Asp Gln Leu Thr Leu Asn Glu Met Ala Val
                165                 170                 175

ATA GCC GGG CTG CCG AAA GCG CCT TCC ACC TTC AAC CCG CTC TAC TCG      576
Ile Ala Gly Leu Pro Lys Ala Pro Ser Thr Phe Asn Pro Leu Tyr Ser
            180                 185                 190

ATG GAT CGT GCC GTC GCG CGG CGT AAC GTC GTG CTG TCG CGG ATG CTG      624
Met Asp Arg Ala Val Ala Arg Arg Asn Val Val Leu Ser Arg Met Leu
        195                 200                 205

GAT GAA GGG TAT ATC ACC CAA CAA CAG TTC GAT CAG ACA CGC ACT GAG      672
Asp Glu Gly Tyr Ile Thr Gln Gln Gln Phe Asp Gln Thr Arg Thr Glu
    210                 215                 220

GCG ATT AAC GCT AAC TAT CAC GCG CCG GAG ATT GCT TTC TCT GCG CCG      720
Ala Ile Asn Ala Asn Tyr His Ala Pro Glu Ile Ala Phe Ser Ala Pro
225                 230                 235                 240

TAC CTG AGC GAA ATG GTG CGC CAG GAG ATG TAT AAC CGT TAT GGC GAA      768
Tyr Leu Ser Glu Met Val Arg Gln Glu Met Tyr Asn Arg Tyr Gly Glu
                245                 250                 255

AGT GCC TAT GAA GAC GGT TAT CGC ATT TAC ACC ACC ATC ACC CGC AAA      816
Ser Ala Tyr Glu Asp Gly Tyr Arg Ile Tyr Thr Thr Ile Thr Arg Lys
            260                 265                 270

GTG CAG CAG GCC GCG CAG CAG GCG GTA CGT AAT AAC GTG CTG GAC TAC      864
Val Gln Gln Ala Ala Gln Gln Ala Val Arg Asn Asn Val Leu Asp Tyr
        275                 280                 285

GAC ATG CGC CAC GGC TAT CGC GGC CCG GCA AAT GTG CTG TGG AAA GTG      912
Asp Met Arg His Gly Tyr Arg Gly Pro Ala Asn Val Leu Trp Lys Val
    290                 295                 300

GGC GAG TCG GCG TGG GAT AAC AAC AAG ATT ACC GAT ACG CTG AAG GCG      960
Gly Glu Ser Ala Trp Asp Asn Asn Lys Ile Thr Asp Thr Leu Lys Ala
305                 310                 315                 320
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CTG CCA ACC TAT GGT CCG CTG CTG CCT GCC GCA GTC ACC AGC GCC AAT      1008
Leu Pro Thr Tyr Gly Pro Leu Leu Pro Ala Ala Val Thr S r Ala Asn
                325                 330                 335

CCT CAG CAA GCG ACG GCG ATG CTG GCG GAC GGG TCG ACC GTC GCA TTG      1056
Pro Gln Gln Ala Thr Ala Met Leu Ala Asp Gly Ser Thr Val Ala L u
            340                 345                 350

AGT ATG GAA GGC GTT CGC TGG GCG CGT CCT TAC CGT TCG GAT ACT CAG      1104
Ser Met Glu Gly Val Arg Trp Ala Arg Pro Tyr Arg Ser Asp Thr Gln
        355                 360                 365

CAA GGA CCG ACG CCG CGT AAA GTG ACC GAT GTT CTG CAA ACG GGT CAG      1152
Gln Gly Pro Thr Pro Arg Lys Val Thr Asp Val Leu Gln Thr Gly Gln
    370                 375                 380

CAA ATC TGG GTT CGT CAG GTT GGC GAT GCA TGG TGG CTG GCA CAA GTG      1200
Gln Ile Trp Val Arg Gln Val Gly Asp Ala Trp Trp Leu Ala Gln Val
385                 390                 395                 400

CCG GAA GTG AAC TCG GCG CTG GTG TCG ATC AAT CCG CAA AAC GGT GCC      1248
Pro Glu Val Asn Ser Ala Leu Val Ser Ile Asn Pro Gln Asn Gly Ala
                405                 410                 415

GTT ATG GCG CTG GTC GGT GGC TTT GAT TTC AAT CAG AGC AAG TTT AAC      1296
Val Met Ala Leu Val Gly Gly Phe Asp Phe Asn Gln Ser Lys Phe Asn
            420                 425                 430

CGC GCC ACC CAG GCA CTG CGT CAG GTG GGT TCC AAC ATC AAA CCG TTC      1344
Arg Ala Thr Gln Ala Leu Arg Gln Val Gly Ser Asn Ile Lys Pro Phe
        435                 440                 445

CTC TAC ACC GCG GCG ATG GAT AAA GGT CTG ACG CTG GCA AGT ATG TTG      1392
Leu Tyr Thr Ala Ala Met Asp Lys Gly Leu Thr Leu Ala Ser Met Leu
    450                 455                 460

AAC GAT GTG CCA ATT TCT CGC TGG GAT GCA AGT GCC GGT TCT GAC TGG      1440
Asn Asp Val Pro Ile Ser Arg Trp Asp Ala Ser Ala Gly Ser Asp Trp
465                 470                 475                 480

CAG CCG AAG AAC TCA CCA CCG CAG TAT GCT GGT CCA ATT CGC TTA CGT      1488
Gln Pro Lys Asn Ser Pro Pro Gln Tyr Ala Gly Pro Ile Arg Leu Arg
                485                 490                 495

CAG GGG CTG GGT CAG TCG AAA AAC GTG GTG ATG GTA CGC GCA ATG CGG      1536
Gln Gly Leu Gly Gln Ser Lys Asn Val Val Met Val Arg Ala Met Arg
            500                 505                 510

GCG ATG GGC GTC GAC TAC GCT GCA GAA TAT CTG CAA CGC TTC GGC TTC      1584
Ala Met Gly Val Asp Tyr Ala Ala Glu Tyr Leu Gln Arg Phe Gly Phe
        515                 520                 525

CCG GCA CAA AAC ATT GTC CAC ACC GAA TCG CTG GCG CTG GGT TCA GCG      1632
Pro Ala Gln Asn Ile Val His Thr Glu Ser Leu Ala Leu Gly Ser Ala
    530                 535                 540

TCC TTC ACC CCA ATG CAG GTG GCG CGC GGC TAC GCG GTC ATG GCG AAC      1680
Ser Phe Thr Pro Met Gln Val Ala Arg Gly Tyr Ala Val Met Ala Asn
545                 550                 555                 560

GGC GGC TTC CTG GTG GAC CCG TGG TTT ATC AGC AAA ATT GAA AAC GAT      1728
Gly Gly Phe Leu Val Asp Pro Trp Phe Ile Ser Lys Ile Glu Asn Asp
                565                 570                 575

CAG GGC GGC GTG ATT TTC GAA GCG AAA CCG AAA GTA GCC TGC CCG GAA      1776
Gln Gly Gly Val Il  Phe Glu Ala Lys Pro Lys Val Ala Cys Pro Glu
            580                 585                 590
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TGC GAT ATT CCG GTG ATT TAC GGT GAT ACG CAG AAA TCG AAC GTG CTG      1824
Cys Asp Ile Pro Val Ile Tyr Gly Asp Thr Gln Lys Ser Asn Val Leu
        595                 600                 605

GAA AAT AAC GAT GTT GAA GAT GTC GCT ATC TCC CGC GAG CAG CAG AAT      1872
Glu Asn Asn Asp Val Glu Asp Val Ala Ile Ser Arg Glu Gln Gln Asn
    610                 615                 620

GTT TCT GTA CCA ATG CCG CAG CTG GAG CAG GCA AAT CAG GCG TTA GTG      1920
Val Ser Val Pro Met Pro Gln Leu Glu Gln Ala Asn Gln Ala Leu Val
625                 630                 635                 640

GCG AAG ACT GGC GCG CAG GAG TAC GCA CCG CAC GTC ATC AAC ACT CCG      1968
Ala Lys Thr Gly Ala Gln Glu Tyr Ala Pro His Val Ile Asn Thr Pro
                645                 650                 655

CTG GCA TTC CTG ATT AAG AGT GCT TTG AAC ACC AAT ATC TTT GGT GAG      2016
Leu Ala Phe Leu Ile Lys Ser Ala Leu Asn Thr Asn Ile Phe Gly Glu
            660                 665                 670

CCA GGC TGG CAG GGT ACT GGC TGG CGT GCA GGT CGT GAT TTG CAG CGT      2064
Pro Gly Trp Gln Gly Thr Gly Trp Arg Ala Gly Arg Asp Leu Gln Arg
        675                 680                 685

CGC GAT ATC GGC GGG AAA ACC GGG ACC ACT AAC AGT TCG AAA GAT GCG      2112
Arg Asp Ile Gly Gly Lys Thr Gly Thr Thr Asn Ser Ser Lys Asp Ala
    690                 695                 700

TGG TTC TCG GGT TAC GGT CCG GGC GTT GTG ACC TCG GTC TGG ATT GGC      2160
Trp Phe Ser Gly Tyr Gly Pro Gly Val Val Thr Ser Val Trp Ile Gly
705                 710                 715                 720

TTT GAT GAT CAC CGT CGT AAT CTC GGT CAT ACA ACG GCT TCC GGA GCG      2208
Phe Asp Asp His Arg Arg Asn Leu Gly His Thr Thr Ala Ser Gly Ala
                725                 730                 735

ATT AAA GAT CAG ATC TCA GGT TAC GAA GGC GGT GCC AAG AGT GCC CAG      2256
Ile Lys Asp Gln Ile Ser Gly Tyr Glu Gly Gly Ala Lys Ser Ala Gln
            740                 745                 750

CCT GCA TGG GAC GCT TAT ATG AAA GCC GTT CTT GAA GGT GTG CCG GAG      2304
Pro Ala Trp Asp Ala Tyr Met Lys Ala Val Leu Glu Gly Val Pro Glu
        755                 760                 765

CAG CCG CTG ACG CCG CCA CCG GGT ATT GTG ACG GTG AAT ATC GAT CGC      2352
Gln Pro Leu Thr Pro Pro Pro Gly Ile Val Thr Val Asn Ile Asp Arg
    770                 775                 780

AGC ACC GGG CAG TTA GCT AAT GGT GGC AAC AGC CGC GAA GAG TAT TTC      2400
Ser Thr Gly Gln Leu Ala Asn Gly Gly Asn Ser Arg Glu Glu Tyr Phe
785                 790                 795                 800

ATC GAA GGT ACG CAG CCG ACA CAA CAG GCA GTG CAC GAG GTG GGA ACG      2448
Ile Glu Gly Thr Gln Pro Thr Gln Gln Ala Val His Glu Val Gly Thr
                805                 810                 815

ACC ATT ATC GAT AAT GGC GAG GCA CAG GAA TTG TTG TG                   2487
Thr Ile Ile Asp Asn Gly Glu Ala Gln Glu Leu Leu
            820                 825
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 828 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Leu Tyr Arg Tyr Ile Glu Pro Gln Leu Pro Asp Val Ala Thr
1 5 10 15

Leu Lys Asp Val Arg Leu Gln Ile Pro Met Gln Ile Tyr Ser Ala Asp
20 25 30

Gly Glu Leu Ile Ala Gln Tyr Gly Glu Lys Arg Arg Ile Pro Val Thr
35 40 45

Leu Asp Gln Ile Pro Pro Glu Met Val Lys Ala Phe Ile Ala Thr Glu
50 55 60

Asp Ser Arg Phe Tyr Glu His His Gly Val Asp Pro Val Gly Ile Phe
65 70 75 80

Arg Ala Ala Ser Val Ala Leu Phe Ser Gly His Ala Ser Gln Gly Ala
85 90 95

Ser Thr Ile Thr Gln Gln Leu Ala Arg Asn Phe Phe Leu Ser Pro Glu
100 105 110

Arg Thr Leu Met Arg Lys Ile Lys Glu Val Phe Leu Ala Ile Arg Ile
115 120 125

Glu Gln Leu Leu Thr Lys Asp Glu Ile Leu Glu Leu Tyr Leu Asn Lys
130 135 140

Ile Tyr Leu Gly Tyr Arg Ala Tyr Gly Val Gly Ala Ala Ala Gln Val
145 150 155 160

Tyr Phe Gly Lys Thr Val Asp Gln Leu Thr Leu Asn Glu Met Ala Val
165 170 175

Ile Ala Gly Leu Pro Lys Ala Pro Ser Thr Phe Asn Pro Leu Tyr Ser
180 185 190

Met Asp Arg Ala Val Ala Arg Arg Asn Val Val Leu Ser Arg Met Leu
195 200 205

Asp Glu Gly Tyr Ile Thr Gln Gln Gln Phe Asp Gln Thr Arg Thr Glu
210 215 220

Ala Ile Asn Ala Asn Tyr His Ala Pro Glu Ile Ala Phe Ser Ala Pro
225 230 235 240

Tyr Leu Ser Glu Met Val Arg Gln Glu Met Tyr Asn Arg Tyr Gly Glu
245 250 255

Ser Ala Tyr Glu Asp Gly Tyr Arg Ile Tyr Thr Thr Ile Thr Arg Lys
260 265 270

Val Gln Gln Ala Ala Gln Gln Ala Val Arg Asn Asn Val Leu Asp Tyr
275 280 285

Asp Met Arg His Gly Tyr Arg Gly Pro Ala Asn Val Leu Trp Lys Val
290 295 300

Gly Glu Ser Ala Trp Asp Asn Asn Lys Ile Thr Asp Thr Leu Lys Ala
305 310 315 320

Leu Pro Thr Tyr Gly Pro Leu Leu Pro Ala Ala Val Thr Ser Ala Asn
325 330 335

Pro Gln Gln Ala Thr Ala Met Leu Ala Asp Gly S r Thr Val Ala Leu
340 345 350

Ser Met Glu Gly Val Arg Trp Ala Arg Pro Tyr Arg Ser Asp Thr Gln
355 360 365

Gln Gly Pro Thr Pro Arg Lys Val Thr Asp Val Leu Gln Thr Gly Gln
370 375 380

Gln Ile Trp Val Arg Gln Val Gly Asp Ala Trp Trp Leu Ala Gln Val
385 390 395 400

Pro Glu Val Asn Ser Ala Leu Val Ser Ile Asn Pro Gln Asn Gly Ala
405 410 415

Val Met Ala Leu Val Gly Gly Phe Asp Phe Asn Gln Ser Lys Phe Asn
420 425 430

Arg Ala Thr Gln Ala Leu Arg Gln Val Gly Ser Asn Ile Lys Pro Phe
435 440 445

Leu Tyr Thr Ala Ala Met Asp Lys Gly Leu Thr Leu Ala Ser Met Leu
450 455 460

Asn Asp Val Pro Ile Ser Arg Trp Asp Ala Ser Ala Gly Ser Asp Trp
465 470 475 480

Gln Pro Lys Asn Ser Pro Pro Gln Tyr Ala Gly Pro Ile Arg Leu Arg
485 490 495

Gln Gly Leu Gly Gln Ser Lys Asn Val Val Met Val Arg Ala Met Arg
500 505 510

Ala Met Gly Val Asp Tyr Ala Ala Glu Tyr Leu Gln Arg Phe Gly Phe
515 520 525

Pro Ala Gln Asn Ile Val His Thr Glu Ser Leu Ala Leu Gly Ser Ala
530 535 540

Ser Phe Thr Pro Met Gln Val Ala Arg Gly Tyr Ala Val Met Ala Asn
545 550 555 560

Gly Gly Phe Leu Val Asp Pro Trp Phe Ile Ser Lys Ile Glu Asn Asp
565 570 575

Gln Gly Gly Val Ile Phe Glu Ala Lys Pro Lys Val Ala Cys Pro Glu
580 585 590

Cys Asp Ile Pro Val Ile Tyr Gly Asp Thr Gln Lys Ser Asn Val Leu
595 600 605

Glu Asn Asn Asp Val Glu Asp Val Ala Ile Ser Arg Glu Gln Gln Asn
610 615 620

Val Ser Val Pro Met Pro Gln Leu Glu Gln Ala Asn Gln Ala Leu Val
625 630 635 640

Ala Lys Thr Gly Ala Gln Glu Tyr Ala Pro His Val Ile Asn Thr Pro
645 650 655

Leu Ala Phe Leu Ile Lys Ser Ala Leu Asn Thr Asn Ile Phe Gly Glu
660 665 670

Pro Gly Trp Gln Gly Thr Gly Trp Arg Ala Gly Arg Asp Leu Gln Arg
675 680 685

Arg Asp Ile Gly Gly Lys Thr Gly Thr Thr Asn Ser Ser Lys Asp Ala
690 695 700

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Trp Phe Ser Gly Tyr Gly Pro Gly Val Val Thr Ser Val Trp Ile Gly
705                 710                 715                 720

Phe Asp Asp His Arg Arg Asn Leu Gly His Thr Thr Ala Ser Gly Ala
                725                 730                 735

Ile Lys Asp Gln Ile Ser Gly Tyr Glu Gly Gly Ala Lys Ser Ala Gln
            740                 745                 750

Pro Ala Trp Asp Ala Tyr Met Lys Ala Val Leu Glu Gly Val Pro Glu
        755                 760                 765

Gln Pro Leu Thr Pro Pro Pro Gly Ile Val Thr Val Asn Ile Asp Arg
    770                 775                 780

Ser Thr Gly Gln Leu Ala Asn Gly Gly Asn Ser Arg Glu Glu Tyr Phe
785                 790                 795                 800

Ile Glu Gly Thr Gln Pro Thr Gln Gln Ala Val His Glu Val Gly Thr
                805                 810                 815

Thr Ile Ile Asp Asn Gly Glu Ala Gln Glu Leu Leu
            820                 825
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2472 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: DH5 alpha

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: PCR cloning
(B) CLONE: pARC 0556 Soluble PBP 1B

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2472

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..2469

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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ATG GCC GGG AAT GAC CGC GAG CCA ATT GGA CGC AAA GGG AAA CCG ACG        48
Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
  1               5                  10                  15

CGT CCG GTC AAA CAA AAG GTA AGC CGT CGT CGT TAC GAA GAT GAC GAT        96
Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
            20                  25                  30

GAT TAC GAC GAT TAT GAT GAC TAT GAG GAT GAA GAA CCG ATG CCG CGC       144
Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
        35                  40                  45
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AAA GGT AAG GGC AAA GGC AAA GGG CGT AAG CCT CGT GGC AAA CGC GGC        192
Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
     50                  55                  60

TCG ATC GAT CAA AAA ATT CGT AGC CGT ATT GAT GGC AAG GTC TGG CAA        240
Ser Ile Asp Gln Lys Ile Arg Ser Arg Ile Asp Gly Lys Val Trp Gln
 65                  70                  75                  80

CTC GCT GCG GCA GTT TAT GGC CGA ATG GTC AAT CTT GAG CCA GAC ATG        288
Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn Leu Glu Pro Asp Met
                 85                  90                  95

ACC ATC AGC AAG AAC GAG ATG GTG AAG CTG CTG GAG GCG ACC CAG TAT        336
Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu Glu Ala Thr Gln Tyr
            100                 105                 110

CGT CAG GTG TCG AAA ATG ACC CGT CCT GGC GAA TTT ACC GTG CAG GCC        384
Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu Phe Thr Val Gln Ala
        115                 120                 125

AAC AGC ATT GAG ATG ATT CGC CGT CCG TTT GAT TTC CCG GAC AGT AAA        432
Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp Phe Pro Asp Ser Lys
    130                 135                 140

GAA GGA CAG GTG CGC GCG CGT CTG ACC TTT GAT GGC GAT CAT CTG GCG        480
Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp Gly Asp His Leu Ala
145                 150                 155                 160

ACG ATC GTC AAT ATG GAG AAC AAC CGT CAG TTC GGT TTC TTC CGT CTT        528
Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe Gly Phe Phe Arg Leu
                165                 170                 175

GAT CCG CGT CTG ATC ACC ATG ATC TCT TCG CCA AAC GGT GAG CAG CGT        576
Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro Asn Gly Glu Gln Arg
            180                 185                 190

CTG TTT GTG CCG CGC AGT GGT TTC CCG GAT TTG CTG GTG GAT ACT TTG        624
Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu Leu Val Asp Thr Leu
        195                 200                 205

CTG GCG ACA GAA GAC CGT CAT TTT TAC GAG CAT GAT GGA ATC AGT CTC        672
Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His Asp Gly Ile Ser Leu
    210                 215                 220

TAC TCA ATC GGA CGT GCG GTG CTG GCA AAC CTG ACC GCC GGA CGC ACG        720
Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu Thr Ala Gly Arg Thr
225                 230                 235                 240

GTA CAG GGT GCG AGT ACG CTG ACG CAA CAG CTG GTG AAA AAC CTG TTC        768
Val Gln Gly Ala Ser Thr Leu Thr Gln Gln Leu Val Lys Asn Leu Phe
                245                 250                 255

CTC TCC AGC GAG CGT TCT TAC TGG CGT AAA GCG AAC GAA GCT TAC ATG        816
Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala Asn Glu Ala Tyr Met
            260                 265                 270

GCG CTG ATC ATG GAC GCG CGT TAC AGC AAA GAC CGT ATT CTT GAG CTG        864
Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp Arg Ile Leu Glu Leu
        275                 280                 285

TAT ATG AAC GAG GTG TAT CTC GGT CAG AGC GGC GAC AAC GAA ATC CGC        912
Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly Asp Asn Glu Ile Arg
    290                 295                 300

GGC TTC CCG CTG GCA AGC TTG TAT TAC TTT GGT CGC CCG GTA GAA GAG        960
Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly Arg Pro Val Glu Glu
305                 310                 315                 320
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CTA AGC CTC GAC CAG CAG GCG CTG TTA GTC GGT ATG GTG AAA GGG GCG      1008
L u Ser Leu Asp Gln Gln Ala Leu Leu Val Gly Met Val Lys Gly Ala
                325                 330                 335

TCC ATC TAC AAC CCG TGG CGT AAC CCA AAA CTG GCG CTG GAG CGA CGT      1056
S r Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu Ala Leu Glu Arg Arg
            340                 345                 350

AAT CTG GTG CTG CGT CTG CTG CAA CAG CAA CAG ATT ATT GAT CAA GAA      1104
Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Gln Ile Ile Asp Gln Glu
        355                 360                 365

CTC TAT GAC ATG TTG AGT GCC CGT CCG CTG GGG GTT CAG CCG CGC GGT      1152
Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly Val Gln Pro Arg Gly
    370                 375                 380

GGG GTG ATC TCT CCT CAG CCA GCC TTT ATG CAA CTG GTG CGT CAG GAG      1200
Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln Leu Val Arg Gln Glu
385                 390                 395                 400

CTG CAG GCA AAA CTG GGC GAT AAG GTA AAA GAT CTC TCC GGC GTG AAG      1248
Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp Leu Ser Gly Val Lys
                405                 410                 415

ATC TTC ACT ACC TTT GAC TCG GTG GCC CAG GAC GCG GCA GAA AAA GCC      1296
Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp Ala Ala Glu Lys Ala
            420                 425                 430

GCC GTG GAA GGC ATT CCG GCA CTG AAG AAA CAG CGT AAG TTG AGC GAT      1344
Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln Arg Lys Leu Ser Asp
        435                 440                 445

CTT GAA ACT GCG ATT GTG GTC GTC GAC CGC TTT AGT GGT GAA GTT CGT      1392
Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe Ser Gly Glu Val Arg
    450                 455                 460

GCG ATG GTC GGA GGT TCT GAG CCG CAG TTT GCG GGC TAC AAC CGT GCG      1440
Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala Gly Tyr Asn Arg Ala
465                 470                 475                 480

ATG CAG GCG CGT CGT TCG ATT GGT TCC CTT GCA AAA CCA GCG ACT TAT      1488
Met Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala Lys Pro Ala Thr Tyr
                485                 490                 495

CTG ACG GCC TTA AGC CAG CCG AAA ATC TAT CGT CTG AAT ACG TGG ATT      1536
Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg Leu Asn Thr Trp Ile
            500                 505                 510

GCG GAT GCG CCA ATT GCG CTG CGT CAG CCG AAT GGC CAG GTC TGG TCA      1584
Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn Gly Gln Val Trp Ser
        515                 520                 525

CCG CAG AAT GAT GAC CGT CGT TAT AGC GAA AGC GGC AGA GTG ATG CTG      1632
Pro Gln Asn Asp Asp Arg Arg Tyr Ser Glu Ser Gly Arg Val Met Leu
    530                 535                 540

GTG GAT GCG TTG ACC CGT TCG ATG AAC GTG CCG ACG GTA AAT CTG GGG      1680
Val Asp Ala Leu Thr Arg Ser Met Asn Val Pro Thr Val Asn Leu Gly
545                 550                 555                 560

ATG GCG CTG GGG CTG CCT GCG GTT ACG GAG ACC TGG ATT AAA CTG GGC      1728
Met Ala Leu Gly Leu Pro Ala Val Thr Glu Thr Trp Ile Lys Leu Gly
                565                 570                 575

GTA CCG AAA GAT CAG TTG CAT CCG GTT CCG GCA ATG CTG CTG GGG GCG      1776
Val Pro Lys Asp Gln Leu His Pro Val Pro Ala Met Leu Leu Gly Ala
            580                 585                 590
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TTG AAC TTA ACG CCA ATC GAA GTG GCG CAG GCA TTC CAG ACC ATC GCC      1824
Leu Asn Leu Thr Pro Ile Glu Val Ala Gln Ala Phe Gln Thr Ile Ala
        595                 600                 605

AGC GGT GGT AAC CGT GCA CCG CTT TCT GCG CTG CGT TCG GTA ATC GCG      1872
Ser Gly Gly Asn Arg Ala Pro Leu Ser Ala Leu Arg Ser Val Ile Ala
    610                 615                 620

GAA GAT GGC AAA GTG CTG TAT CAG AGC TTC CCG CAG GCG GAA CGC GCT      1920
Glu Asp Gly Lys Val Leu Tyr Gln Ser Phe Pro Gln Ala Glu Arg Ala
625                 630                 635                 640

GTT CCG GCG CAG GCG GCG TAT CTG ACA CTA TGG ACC ATG CAG CAG GTG      1968
Val Pro Ala Gln Ala Ala Tyr Leu Thr Leu Trp Thr Met Gln Gln Val
                645                 650                 655

GTA CAA CGC GGT ACG GGT CGT CAG CTT GGG GCG AAA TAC CCG AAC CTG      2016
Val Gln Arg Gly Thr Gly Arg Gln Leu Gly Ala Lys Tyr Pro Asn Leu
            660                 665                 670

CAT CTG GCA GGG AAA ACA GGG ACT ACC AAC AAT AAC GTA GAT ACC TGG      2064
His Leu Ala Gly Lys Thr Gly Thr Thr Asn Asn Asn Val Asp Thr Trp
        675                 680                 685

TTT GCG GGC ATT GAC GGC AGC ACG GTG ACC ATC ACC TGG GTC GGC CGT      2112
Phe Ala Gly Ile Asp Gly Ser Thr Val Thr Ile Thr Trp Val Gly Arg
    690                 695                 700

GAT AAC AAC CAG CCG ACC AAA CTG TAT GGT GCC AGC GGG GCA ATG TCG      2160
Asp Asn Asn Gln Pro Thr Lys Leu Tyr Gly Ala Ser Gly Ala Met Ser
705                 710                 715                 720

ATT TAT CAG CGT TAT CTG GCT AAC CAG ACG CCA ACG CCG CTG AAT CTT      2208
Ile Tyr Gln Arg Tyr Leu Ala Asn Gln Thr Pro Thr Pro Leu Asn Leu
                725                 730                 735

GTT CCG CCA GAA GAT ATT GCA GAT ATG GGC GTG GAC TAC GAC GGC AAC      2256
Val Pro Pro Glu Asp Ile Ala Asp Met Gly Val Asp Tyr Asp Gly Asn
            740                 745                 750

TTT GTT TGC AGC GGT GGC ATG CGT ATC TTG CCG GTC TGG ACC AGC GAT      2304
Phe Val Cys Ser Gly Gly Met Arg Ile Leu Pro Val Trp Thr Ser Asp
        755                 760                 765

CCG CAA TCG CTG TGC CAG CAG AGC GAG ATG CAG CAG CAG CCG TCA GGC      2352
Pro Gln Ser Leu Cys Gln Gln Ser Glu Met Gln Gln Gln Pro Ser Gly
    770                 775                 780

AAT CCG TTT GAT CAG TCT TCT CAG CCG CAG CAA CAG CCG CAA CAG CAA      2400
Asn Pro Phe Asp Gln Ser Ser Gln Pro Gln Gln Gln Pro Gln Gln Gln
785                 790                 795                 800

CCT GCT CAG CAA GAG CAG AAA GAC AGC GAC GGT GTA GCC GGT TGG ATC      2448
Pro Ala Gln Gln Glu Gln Lys Asp Ser Asp Gly Val Ala Gly Trp Ile
                805                 810                 815

AAG GAT ATG TTT GGT AGT AAT TA                                      2472
Lys Asp Met Phe Gly Ser Asn
            820
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 823 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: prot in

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
M t Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
  1               5                  10                  15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
            20                  25                  30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
        35                  40                  45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
    50                  55                  60

Ser Ile Asp Gln Lys Ile Arg Ser Arg Ile Asp Gly Lys Val Trp Gln
65                  70                  75                  80

Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn Leu Glu Pro Asp Met
                85                  90                  95

Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu Glu Ala Thr Gln Tyr
            100                 105                 110

Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu Phe Thr Val Gln Ala
        115                 120                 125

Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp Phe Pro Asp Ser Lys
    130                 135                 140

Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp Gly Asp His Leu Ala
145                 150                 155                 160

Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe Gly Phe Phe Arg Leu
                165                 170                 175

Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro Asn Gly Glu Gln Arg
            180                 185                 190

Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu Leu Val Asp Thr Leu
        195                 200                 205

Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His Asp Gly Ile Ser Leu
    210                 215                 220

Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu Thr Ala Gly Arg Thr
225                 230                 235                 240

Val Gln Gly Ala Ser Thr Leu Thr Gln Gln Leu Val Lys Asn Leu Phe
                245                 250                 255

Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala Asn Glu Ala Tyr Met
            260                 265                 270

Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp Arg Ile Leu Glu Leu
        275                 280                 285

Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly Asp Asn Glu Ile Arg
    290                 295                 300

Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly Arg Pro Val Glu Glu
305                 310                 315                 320

Leu Ser Leu Asp Gln Gln Ala Leu Leu Val Gly Met Val Lys Gly Ala
                325                 330                 335
```

Ser Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu Ala Leu Glu Arg Arg
340 345 350

Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Ile Ile Asp Gln Glu
355 360 365

Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly Val Gln Pro Arg Gly
370 375 380

Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln Leu Val Arg Gln Glu
385 390 395 400

Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp Leu Ser Gly Val Lys
405 410 415

Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp Ala Ala Glu Lys Ala
420 425 430

Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln Arg Lys Leu Ser Asp
435 440 445

Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe Ser Gly Glu Val Arg
450 455 460

Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala Gly Tyr Asn Arg Ala
465 470 475 480

Met Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala Lys Pro Ala Thr Tyr
485 490 495

Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg Leu Asn Thr Trp Ile
500 505 510

Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn Gly Gln Val Trp Ser
515 520 525

Pro Gln Asn Asp Asp Arg Arg Tyr Ser Glu Ser Gly Arg Val Met Leu
530 535 540

Val Asp Ala Leu Thr Arg Ser Met Asn Val Pro Thr Val Asn Leu Gly
545 550 555 560

Met Ala Leu Gly Leu Pro Ala Val Thr Glu Thr Trp Ile Lys Leu Gly
565 570 575

Val Pro Lys Asp Gln Leu His Pro Val Pro Ala Met Leu Leu Gly Ala
580 585 590

Leu Asn Leu Thr Pro Ile Glu Val Ala Gln Ala Phe Gln Thr Ile Ala
595 600 605

Ser Gly Gly Asn Arg Ala Pro Leu Ser Ala Leu Arg Ser Val Ile Ala
610 615 620

Glu Asp Gly Lys Val Leu Tyr Gln Ser Phe Pro Gln Ala Glu Arg Ala
625 630 635 640

Val Pro Ala Gln Ala Ala Tyr Leu Thr Leu Trp Thr Met Gln Gln Val
645 650 655

Val Gln Arg Gly Thr Gly Arg Gln Leu Gly Ala Lys Tyr Pro Asn Leu
660 665 670

His Leu Ala Gly Lys Thr Gly Thr Thr Asn Asn Asn Val Asp Thr Trp
675 680 685

Phe Ala Gly Ile Asp Gly Ser Thr Val Thr Ile Thr Trp Val Gly Arg
690 695 700

```
Asp Asn Asn Gln Pro Thr Lys Leu Tyr Gly Ala Ser Gly Ala Met S r
705                 710                 715                 720

Ile Tyr Gln Arg Tyr Leu Ala Asn Gln Thr Pro Thr Pro L u Asn Leu
                725                 730                 735

Val Pro Pro Glu Asp Ile Ala Asp Met Gly Val Asp Tyr Asp Gly Asn
            740                 745                 750

Phe Val Cys Ser Gly Gly Met Arg Ile Leu Pro Val Trp Thr Ser Asp
        755                 760                 765

Pro Gln Ser Leu Cys Gln Gln Ser Glu Met Gln Gln Gln Pro Ser Gly
    770                 775                 780

Asn Pro Phe Asp Gln Ser Ser Gln Pro Gln Gln Gln Pro Gln Gln Gln
785                 790                 795                 800

Pro Ala Gln Gln Glu Gln Lys Asp Ser Asp Gly Val Ala Gly Trp Ile
                805                 810                 815

Lys Asp Met Phe Gly Ser Asn
            820
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2049 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptococcus pneumoniae
(B) STRAIN: PM 1

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: PCR cloning
(B) CLONE: pARC 0512 Soluble PBP 1A del 38

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2049

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..2046

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ATG GCT CCT AGC CTA TCC GAG AGT AAA CTA GTT GCA ACA ACT TCT AGT        48
Met Ala Pro Ser Leu Ser Glu Ser Lys Leu Val Ala Thr Thr Ser Ser
 1               5                  10                  15

AAA ATC TAC GAC AAT AAA AAT CAA CTC ATT GCT GAC TTG GGT TCT GAA        96
Lys Ile Tyr Asp Asn Lys Asn Gln Leu Ile Ala Asp Leu Gly Ser Glu
            20                  25                  30

CGC CGC GTC AAT GCC CAA GCT AAT GAT ATT CCC ACA GAT TTG GTT AAG       144
Arg Arg Val Asn Ala Gln Ala Asn Asp Ile Pro Thr Asp Leu Val Lys
        35                  40                  45
```

```
GCA ATC GTT TCT ATC GAA GAC CAT CGC TTC TTC GAC CAC AGG GGG ATT        192
Ala Ile Val Ser Ile Glu Asp His Arg Phe Ph  Asp His Arg Gly Il
     50                  55                  60

GAT ACC ATC CGT ATC CTG GGA GCT TTC TTG CGC AAT CTG CAA AGC AAT        240
Asp Thr Ile Arg Ile Leu Gly Ala Phe Leu Arg Asn Leu Gln Ser Asn
 65                  70                  75                  80

TCC CTC CAA GGT GGA TCA GCT CTC ACT CAA CAG TTG ATT AAG TTG ACT        288
Ser Leu Gln Gly Gly Ser Ala Leu Thr Gln Gln Leu Ile Lys Leu Thr
                 85                  90                  95

TAC TTT TCA ACT TCG ACT TCC GAC CAG ACT ATT TCT CGT AAG GCT CAG        336
Tyr Phe Ser Thr Ser Thr Ser Asp Gln Thr Ile Ser Arg Lys Ala Gln
            100                 105                 110

GAA GCT TGG TTA GCG ATT CAG TTA GAA CAA AAA GCA ACC AAG CAA GAA        384
Glu Ala Trp Leu Ala Ile Gln Leu Glu Gln Lys Ala Thr Lys Gln Glu
        115                 120                 125

ATC TTG ACC TAC TAT ATA AAT AAG GTC TAC ATG TCT AAT GGG AAC TAT        432
Ile Leu Thr Tyr Tyr Ile Asn Lys Val Tyr Met Ser Asn Gly Asn Tyr
    130                 135                 140

GGA ATG CAG ACA GCA GCT CAA AAC TAC TAT GGT AAA GAC CTC AAT AAT        480
Gly Met Gln Thr Ala Ala Gln Asn Tyr Tyr Gly Lys Asp Leu Asn Asn
145                 150                 155                 160

TTA AGT TTA CCT CAG TTA GCC TTG CTG GCT GGA ATG CCT CAG GCA CCA        528
Leu Ser Leu Pro Gln Leu Ala Leu Leu Ala Gly Met Pro Gln Ala Pro
                165                 170                 175

AAC CAA TAT GAC CCC TAT TCA CAT CCA GAA GCA GCC CAA GAC CGC CGA        576
Asn Gln Tyr Asp Pro Tyr Ser His Pro Glu Ala Ala Gln Asp Arg Arg
            180                 185                 190

AAC TTG GTC TTA TCT GAA ATG AAA AAT CAA GGC TAC ATC TCT GCT GAA        624
Asn Leu Val Leu Ser Glu Met Lys Asn Gln Gly Tyr Ile Ser Ala Glu
        195                 200                 205

CAG TAT GAG AAA GCA GTC AAT ACA CCA ATT ACT GAT GGG CTA CAA AGT        672
Gln Tyr Glu Lys Ala Val Asn Thr Pro Ile Thr Asp Gly Leu Gln Ser
    210                 215                 220

CTC AAA TCA GCA AGT AAT TAC CCT GCT TAC ATG GAT AAT TAC CTC AAG        720
Leu Lys Ser Ala Ser Asn Tyr Pro Ala Tyr Met Asp Asn Tyr Leu Lys
225                 230                 235                 240

GAA GTC ATC AAT CAA GTT GAA GAA GAA ACA GGC TAT AAC CTA CTC ACA        768
Glu Val Ile Asn Gln Val Glu Glu Glu Thr Gly Tyr Asn Leu Leu Thr
                245                 250                 255

ACT GGG ATG GAT GTC TAC ACA AAT GTA GAC CAA GAA GCT CAA AAA CAT        816
Thr Gly Met Asp Val Tyr Thr Asn Val Asp Gln Glu Ala Gln Lys His
            260                 265                 270

CTG TGG GAT ATT TAC AAT ACA GAC GAA TAC GTT GCC TAT CCA GAC GAT        864
Leu Trp Asp Ile Tyr Asn Thr Asp Glu Tyr Val Ala Tyr Pro Asp Asp
        275                 280                 285

GAA TTG CAA GTC GCT TCT ACC ATT GTT GAT GTT TCT AAC GGT AAA GTC        912
Glu Leu Gln Val Ala Ser Thr Ile Val Asp Val Ser Asn Gly Lys Val
    290                 295                 300

ATT GCC CAG CTA GGA GCA CGC CAT CAG TCA AGT AAT GTT TCC TTC GGA        960
Ile Ala Gln Leu Gly Ala Arg His Gln Ser Ser Asn Val Ser Phe Gly
305                 310                 315                 320
```

```
ATT AAC CAA GCA GTA GAA ACA AAC CGC GAC TGG GGA TCA ACT ATG AAA      1008
Il  Asn Gln Ala Val Glu Thr Asn Arg Asp Trp Gly Ser Thr Met Lys
                325                 330                 335

CCG ATC ACA GAC TAT GCT CCT GCC TTG GAG TAC GGT GTC TAC GAG TCA      1056
Pro Il  Thr Asp Tyr Ala Pro Ala Leu Glu Tyr Gly Val Tyr Glu Ser
            340                 345                 350

ACT GCC ACT ATC GTT CAC GAT GAG CCC TAT AAC TAC CCT GGG ACA AAT      1104
Thr Ala Thr Ile Val His Asp Glu Pro Tyr Asn Tyr Pro Gly Thr Asn
        355                 360                 365

ACC CCT GTT TAT AAC TGG GAT AGG GGC TAC TTT GGC AAC ATC ACC TTG      1152
Thr Pro Val Tyr Asn Trp Asp Arg Gly Tyr Phe Gly Asn Ile Thr Leu
    370                 375                 380

CAA TAC GCC CTG CAA CAA TCG CGA AAC GTC CCA GCC GTG GAA ACT CTA      1200
Gln Tyr Ala Leu Gln Gln Ser Arg Asn Val Pro Ala Val Glu Thr Leu
385                 390                 395                 400

AAC AAG GTC GGA CTC AAC CGC GCC AAG ACT TTC CTA AAT GGT CTC GGA      1248
Asn Lys Val Gly Leu Asn Arg Ala Lys Thr Phe Leu Asn Gly Leu Gly
                405                 410                 415

ATC GAC TAC CCA AGT ATT CAC TAC TCA AAT GCC ATT TCA AGT AAC ACA      1296
Ile Asp Tyr Pro Ser Ile His Tyr Ser Asn Ala Ile Ser Ser Asn Thr
            420                 425                 430

ACC GAA TCA GAC AAA AAA TAT GGA GCA AGT AGT GAA AAG ATG GCT GCT      1344
Thr Glu Ser Asp Lys Lys Tyr Gly Ala Ser Ser Glu Lys Met Ala Ala
        435                 440                 445

GCT TAC GCT GCC TTT GCA AAT GGT GGA ACT TAC TAT AAA CCA ATG TAT      1392
Ala Tyr Ala Ala Phe Ala Asn Gly Gly Thr Tyr Tyr Lys Pro Met Tyr
    450                 455                 460

ATC CAT AAA GTC GTC TTT AGT GAT GGG AGT GAA AAA GAG TTC TCT AAT      1440
Ile His Lys Val Val Phe Ser Asp Gly Ser Glu Lys Glu Phe Ser Asn
465                 470                 475                 480

GTC GGA ACT CGT GCC ATG AAA GAA ACG ACA GCC TAT ATG ATG ACC GAC      1488
Val Gly Thr Arg Ala Met Lys Glu Thr Thr Ala Tyr Met Met Thr Asp
                485                 490                 495

ATG ATG AAA ACA GTC TTG AGT TAT GGA ACT GGA CGA AAT GCC TAT CTT      1536
Met Met Lys Thr Val Leu Ser Tyr Gly Thr Gly Arg Asn Ala Tyr Leu
            500                 505                 510

GCT TGG CTC CCT CAG GCT GGT AAA ACA GGA ACC TCT AAC TAT ACA GAC      1584
Ala Trp Leu Pro Gln Ala Gly Lys Thr Gly Thr Ser Asn Tyr Thr Asp
        515                 520                 525

GAG GAA ATT GAA AAC CAC ATC AAG ACC TCT CAA TTT GTA GCA CCT GAT      1632
Glu Glu Ile Glu Asn His Ile Lys Thr Ser Gln Phe Val Ala Pro Asp
    530                 535                 540

GAA CTA TTT GCT GGC TAT ACG CGT AAA TAT TCA ATG GCT GTA TGG ACA      1680
Glu Leu Phe Ala Gly Tyr Thr Arg Lys Tyr Ser Met Ala Val Trp Thr
545                 550                 555                 560

GGC TAT TCT AAC CGT CTG ACA CCA CTT GTA GGC AAT GGC CTT ACG GTC      1728
Gly Tyr Ser Asn Arg Leu Thr Pro Leu Val Gly Asn Gly Leu Thr Val
                565                 570                 575

GCT GCC AAA GTT TAC CGC TCT ATG ATG ACC TAC CTG TCT GAA GGA AGC      1776
Ala Ala Lys Val Tyr Arg Ser Met Met Thr Tyr Leu Ser Glu Gly Ser
            580                 585                 590
```

```
AAT CCA GAG GAT TGG AAT ATA CCA GAG GGG CTC TAC AGA AAT GGA GAA      1824
Asn Pro Glu Asp Trp Asn Ile Pro Glu Gly Leu Tyr Arg Asn Gly Glu
        595                 600                 605

TTC GTA TTT AAA AAT GGT GCT CGT TCT ACG TGG AGC TCA CCT GCT CCA      1872
Phe Val Phe Lys Asn Gly Ala Arg Ser Thr Trp Ser Ser Pro Ala Pro
    610                 615                 620

CAA CAA CCC CCA TCA ACT GAA AGT TCA AGC TCA TCA TCA GAT AGT TCA      1920
Gln Gln Pro Pro Ser Thr Glu Ser Ser Ser Ser Ser Ser Asp Ser Ser
625                 630                 635                 640

ACT TCA CAG TCT AGC TCA ACC ACT CCA AGC ACA AAT AAT AGT ACG ACT      1968
Thr Ser Gln Ser Ser Ser Thr Thr Pro Ser Thr Asn Asn Ser Thr Thr
                645                 650                 655

ACC AAT CCT AAC AAT AAT ACG CAA CAA TCA AAT ACA ACC CCT GAT CAA      2016
Thr Asn Pro Asn Asn Asn Thr Gln Gln Ser Asn Thr Thr Pro Asp Gln
            660                 665                 670

CAA AAT CAG AAT CCT CAA CCA GCA CAA CCA TA                           2049
Gln Asn Gln Asn Pro Gln Pro Ala Gln Pro
        675                 680
```

(2: INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 682 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ala Pro Ser Leu Ser Glu Ser Lys Leu Val Ala Thr Thr Ser Ser
  1               5                  10                  15

Lys Ile Tyr Asp Asn Lys Asn Gln Leu Ile Ala Asp Leu Gly Ser Glu
            20                  25                  30

Arg Arg Val Asn Ala Gln Ala Asn Asp Ile Pro Thr Asp Leu Val Lys
        35                  40                  45

Ala Ile Val Ser Ile Glu Asp His Arg Phe Phe Asp His Arg Gly Ile
    50                  55                  60

Asp Thr Ile Arg Ile Leu Gly Ala Phe Leu Arg Asn Leu Gln Ser Asn
65                  70                  75                  80

Ser Leu Gln Gly Gly Ser Ala Leu Thr Gln Gln Leu Ile Lys Leu Thr
                85                  90                  95

Tyr Phe Ser Thr Ser Thr Ser Asp Gln Thr Ile Ser Arg Lys Ala Gln
            100                 105                 110

Glu Ala Trp Leu Ala Ile Gln Leu Glu Gln Lys Ala Thr Lys Gln Glu
        115                 120                 125

Ile Leu Thr Tyr Tyr Ile Asn Lys Val Tyr Met Ser Asn Gly Asn Tyr
    130                 135                 140

Gly Met Gln Thr Ala Ala Gln Asn Tyr Tyr Gly Lys Asp Leu Asn Asn
145                 150                 155                 160

Leu Ser Leu Pro Gln Leu Ala Leu Leu Ala Gly Met Pro Gln Ala Pro
                165                 170                 175
```

```
Asn Gln Tyr Asp Pro Tyr S r His Pro Glu Ala Ala Gln Asp Arg Arg
            180                 185                 190

Asn Leu Val Leu Ser Glu Met Lys Asn Gln Gly Tyr Ile Ser Ala Glu
        195                 200                 205

Gln Tyr Glu Lys Ala Val Asn Thr Pro Ile Thr Asp Gly Leu Gln Ser
    210                 215                 220

Leu Lys Ser Ala Ser Asn Tyr Pro Ala Tyr Met Asp Asn Tyr Leu Lys
225                 230                 235                 240

Glu Val Ile Asn Gln Val Glu Glu Glu Thr Gly Tyr Asn Leu Leu Thr
                245                 250                 255

Thr Gly Met Asp Val Tyr Thr Asn Val Asp Gln Glu Ala Gln Lys His
            260                 265                 270

Leu Trp Asp Ile Tyr Asn Thr Asp Glu Tyr Val Ala Tyr Pro Asp Asp
        275                 280                 285

Glu Leu Gln Val Ala Ser Thr Ile Val Asp Val Ser Asn Gly Lys Val
    290                 295                 300

Ile Ala Gln Leu Gly Ala Arg His Gln Ser Ser Asn Val Ser Phe Gly
305                 310                 315                 320

Ile Asn Gln Ala Val Glu Thr Asn Arg Asp Trp Gly Ser Thr Met Lys
                325                 330                 335

Pro Ile Thr Asp Tyr Ala Pro Ala Leu Glu Tyr Gly Val Tyr Glu Ser
            340                 345                 350

Thr Ala Thr Ile Val His Asp Glu Pro Tyr Asn Tyr Pro Gly Thr Asn
        355                 360                 365

Thr Pro Val Tyr Asn Trp Asp Arg Gly Tyr Phe Gly Asn Ile Thr Leu
    370                 375                 380

Gln Tyr Ala Leu Gln Gln Ser Arg Asn Val Pro Ala Val Glu Thr Leu
385                 390                 395                 400

Asn Lys Val Gly Leu Asn Arg Ala Lys Thr Phe Leu Asn Gly Leu Gly
                405                 410                 415

Ile Asp Tyr Pro Ser Ile His Tyr Ser Asn Ala Ile Ser Ser Asn Thr
            420                 425                 430

Thr Glu Ser Asp Lys Lys Tyr Gly Ala Ser Ser Glu Lys Met Ala Ala
        435                 440                 445

Ala Tyr Ala Ala Phe Ala Asn Gly Gly Thr Tyr Tyr Lys Pro Met Tyr
    450                 455                 460

Ile His Lys Val Val Phe Ser Asp Gly Ser Glu Lys Glu Phe Ser Asn
465                 470                 475                 480

Val Gly Thr Arg Ala Met Lys Glu Thr Thr Ala Tyr Met Met Thr Asp
                485                 490                 495

Met Met Lys Thr Val Leu Ser Tyr Gly Thr Gly Arg Asn Ala Tyr Leu
            500                 505                 510

Ala Trp Leu Pro Gln Ala Gly Lys Thr Gly Thr Ser Asn Tyr Thr Asp
        515                 520                 525

Glu Glu Ile Glu Asn His Ile Lys Thr Ser Gln Phe Val Ala Pro Asp
    530                 535                 540
```

```
Glu Leu Ph  Ala Gly Tyr Thr Arg Lys Tyr Ser M t Ala Val Trp Thr
545                 550                 555                 560

Gly Tyr Ser Asn Arg Leu Thr Pro Leu Val Gly Asn Gly Leu Thr Val
                565                 570                 575

Ala Ala Lys Val Tyr Arg Ser Met Met Thr Tyr Leu Ser Glu Gly Ser
            580                 585                 590

Asn Pro Glu Asp Trp Asn Ile Pro Glu Gly Leu Tyr Arg Asn Gly Glu
        595                 600                 605

Phe Val Phe Lys Asn Gly Ala Arg Ser Thr Trp Ser Ser Pro Ala Pro
    610                 615                 620

Gln Gln Pro Pro Ser Thr Glu Ser Ser Ser Ser Ser Ser Asp Ser Ser
625                 630                 635                 640

Thr Ser Gln Ser Ser Ser Thr Thr Pro Ser Thr Asn Asn Ser Thr Thr
                645                 650                 655

Thr Asn Pro Asn Asn Asn Thr Gln Gln Ser Asn Thr Thr Pro Asp Gln
            660                 665                 670

Gln Asn Gln Asn Pro Gln Pro Ala Gln Pro
        675                 680
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 844 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC0438 PBP 1B QQAA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
1               5                   10                  15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
            20                  25                  30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
        35                  40                  45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
    50                  55                  60

Trp Leu Trp Leu Leu Leu Lys Leu Ala Ile Val Phe Ala Val Leu Ile
65                  70                  75                  80

Ala Ile Tyr Gly Val Tyr Leu Asp Gln Lys Ile Arg Ser Arg Ile Asp
                85                  90                  95

Gly Lys Val Trp Gln Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn
            100                 105                 110
```

Leu Glu Pro Asp Met Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu
115 120 125

Glu Ala Thr Gln Tyr Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu
130 135 140

Phe Thr Val Gln Ala Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp
145 150 155 160

Phe Pro Asp Ser Lys Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp
165 170 175

Gly Asp His Leu Ala Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe
180 185 190

Gly Phe Phe Arg Leu Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro
195 200 205

Asn Gly Glu Gln Arg Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu
210 215 220

Leu Val Asp Thr Leu Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His
225 230 235 240

Asp Gly Ile Ser Leu Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu
245 250 255

Thr Ala Gly Arg Thr Val Gln Gly Ala Ser Thr Leu Thr Ala Ala Leu
260 265 270

Val Lys Asn Leu Phe Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala
275 280 285

Asn Glu Ala Tyr Met Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp
290 295 300

Arg Ile Leu Glu Leu Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly
305 310 315 320

Asp Asn Glu Ile Arg Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly
325 330 335

Arg Pro Val Glu Glu Leu Ser Leu Asp Gln Gln Ala Leu Leu Val Gly
340 345 350

Met Val Lys Gly Ala Ser Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu
355 360 365

Ala Leu Glu Arg Arg Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Gln
370 375 380

Ile Ile Asp Gln Glu Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly
385 390 395 400

Val Gln Pro Arg Gly Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln
405 410 415

Leu Val Arg Gln Glu Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp
420 425 430

Leu Ser Gly Val Lys Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp
435 440 445

Ala Ala Glu Lys Ala Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln
450 455 460

Arg Lys Leu Ser Asp Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe
465 470 475 480

S r Gly Glu Val Arg Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala
485 490 495

Gly Tyr Asn Arg Ala M t Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala
500 505 510

Lys Pro Ala Thr Tyr Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg
515 520 525

Leu Asn Thr Trp Ile Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn
530 535 540

Gly Gln Val Trp Ser Pro Gln Asn Asp Asp Arg Arg Tyr Ser Glu Ser
545 550 555 560

Gly Arg Val Met Leu Val Asp Ala Leu Thr Arg Ser Met Asn Val Pro
565 570 575

Thr Val Asn Leu Gly Met Ala Leu Gly Leu Pro Ala Val Thr Glu Thr
580 585 590

Trp Ile Lys Leu Gly Val Pro Lys Asp Gln Leu His Pro Val Pro Ala
595 600 605

Met Leu Leu Gly Ala Leu Asn Leu Thr Pro Ile Glu Val Ala Gln Ala
610 615 620

Phe Gln Thr Ile Ala Ser Gly Gly Asn Arg Ala Pro Leu Ser Ala Leu
625 630 635 640

Arg Ser Val Ile Ala Glu Asp Gly Lys Val Leu Tyr Gln Ser Phe Pro
645 650 655

Gln Ala Glu Arg Ala Val Pro Ala Gln Ala Ala Tyr Leu Thr Leu Trp
660 665 670

Thr Met Gln Gln Val Val Gln Arg Gly Thr Gly Arg Gln Leu Gly Ala
675 680 685

Lys Tyr Pro Asn Leu His Leu Ala Gly Lys Thr Gly Thr Thr Asn Asn
690 695 700

Asn Val Asp Thr Trp Phe Ala Gly Ile Asp Gly Ser Thr Val Thr Ile
705 710 715 720

Thr Trp Val Gly Arg Asp Asn Asn Gln Pro Thr Lys Leu Tyr Gly Ala
725 730 735

Ser Gly Ala Met Ser Ile Tyr Gln Arg Tyr Leu Ala Asn Gln Thr Pro
740 745 750

Thr Pro Leu Asn Leu Val Pro Pro Glu Asp Ile Ala Asp Met Gly Val
755 760 765

Asp Tyr Asp Gly Asn Phe Val Cys Ser Gly Gly Met Arg Ile Leu Pro
770 775 780

Val Trp Thr Ser Asp Pro Gln Ser Leu Cys Gln Gln Ser Glu Met Gln
785 790 795 800

Gln Gln Pro Ser Gly Asn Pro Phe Asp Gln Ser Ser Gln Pro Gln Gln
805 810 815

Gln Pro Gln Gln Gln Pro Ala Gln Gln Glu Gln Lys Asp Ser Asp Gly
820 825 830

Val Ala Gly Trp Ile Lys Asp Met Phe Gly Ser Asn
835 840

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 844 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC0468 PBP 1B QQLL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
1 5 10 15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
20 25 30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
35 40 45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
50 55 60

Trp Leu Trp Leu Leu Leu Lys Leu Ala Ile Val Phe Ala Val Leu Ile
65 70 75 80

Ala Ile Tyr Gly Val Tyr Leu Asp Gln Lys Ile Arg Ser Arg Ile Asp
85 90 95

Gly Lys Val Trp Gln Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn
100 105 110

Leu Glu Pro Asp Met Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu
115 120 125

Glu Ala Thr Gln Tyr Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu
130 135 140

Phe Thr Val Gln Ala Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp
145 150 155 160

Phe Pro Asp Ser Lys Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp
165 170 175

Gly Asp His Leu Ala Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe
180 185 190

Gly Phe Phe Arg Leu Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro
195 200 205

Asn Gly Glu Gln Arg Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu
210 215 220

Leu Val Asp Thr Leu Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His
225 230 235 240

Asp Gly Ile Ser Leu Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu
245 250 255

Thr Ala Gly Arg Thr Val Gln Gly Ala Ser Thr Leu Thr Leu Leu Leu
260 265 270

Val Lys Asn L u Phe Leu Ser S r Glu Arg Ser Tyr Trp Arg Lys Ala
275 280 285

Asn Glu Ala Tyr Met Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp
290 295 300

Arg Ile Leu Glu Leu Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly
305 310 315 320

Asp Asn Glu Ile Arg Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly
325 330 335

Arg Pro Val Glu Glu Leu Ser Leu Asp Gln Gln Ala Leu Leu Val Gly
340 345 350

Met Val Lys Gly Ala Ser Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu
355 360 365

Ala Leu Glu Arg Arg Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Gln
370 375 380

Ile Ile Asp Gln Glu Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly
385 390 395 400

Val Gln Pro Arg Gly Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln
405 410 415

Leu Val Arg Gln Glu Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp
420 425 430

Leu Ser Gly Val Lys Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp
435 440 445

Ala Ala Glu Lys Ala Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln
450 455 460

Arg Lys Leu Ser Asp Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe
465 470 475 480

Ser Gly Glu Val Arg Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala
485 490 495

Gly Tyr Asn Arg Ala Met Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala
500 505 510

Lys Pro Ala Thr Tyr Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg
515 520 525

Leu Asn Thr Trp Ile Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn
530 535 540

Gly Gln Val Trp Ser Pro Gln Asn Asp Asp Arg Arg Tyr Ser Glu Ser
545 550 555 560

Gly Arg Val Met Leu Val Asp Ala Leu Thr Arg Ser Met Asn Val Pro
565 570 575

Thr Val Asn Leu Gly Met Ala Leu Gly Leu Pro Ala Val Thr Glu Thr
580 585 590

Trp Ile Lys Leu Gly Val Pro Lys Asp Gln Leu His Pro Val Pro Ala
595 600 605

Met Leu Leu Gly Ala L u Asn Leu Thr Pro Ile Glu Val Ala Gln Ala
610 615 620

Phe Gln Thr Ile Ala Ser Gly Gly Asn Arg Ala Pro Leu Ser Ala Leu
625 630 635 640

Arg Ser Val Ile Ala Glu Asp Gly Lys Val Leu Tyr Gln Ser Phe Pro
645 650 655

Gln Ala Glu Arg Ala Val Pro Ala Gln Ala Ala Tyr L u Thr Leu Trp
660 665 670

Thr Met Gln Gln Val Val Gln Arg Gly Thr Gly Arg Gln Leu Gly Ala
675 680 685

Lys Tyr Pro Asn Leu His Leu Ala Gly Lys Thr Gly Thr Thr Asn Asn
690 695 700

Asn Val Asp Thr Trp Phe Ala Gly Ile Asp Gly Ser Thr Val Thr Ile
705 710 715 720

Thr Trp Val Gly Arg Asp Asn Asn Gln Pro Thr Lys Leu Tyr Gly Ala
725 730 735

Ser Gly Ala Met Ser Ile Tyr Gln Arg Tyr Leu Ala Asn Gln Thr Pro
740 745 750

Thr Pro Leu Asn Leu Val Pro Pro Glu Asp Ile Ala Asp Met Gly Val
755 760 765

Asp Tyr Asp Gly Asn Phe Val Cys Ser Gly Gly Met Arg Ile Leu Pro
770 775 780

Val Trp Thr Ser Asp Pro Gln Ser Leu Cys Gln Gln Ser Glu Met Gln
785 790 795 800

Gln Gln Pro Ser Gly Asn Pro Phe Asp Gln Ser Ser Gln Pro Gln Gln
805 810 815

Gln Pro Gln Gln Gln Pro Ala Gln Gln Glu Gln Lys Asp Ser Asp Gly
820 825 830

Val Ala Gly Trp Ile Lys Asp Met Phe Gly Ser Asn
835 840

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 836 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC0469 PBP 1B del 8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
1 5 10 15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
20 25 30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
35 40 45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
50 55 60

Trp Leu Trp Leu L u Leu Lys Leu Ala Il Val Phe Ala Val Leu Ile
65 70 75 80

Ala Ile Tyr Gly Val Tyr Leu Asp Gln Lys Ile Arg Ser Arg Ile Asp
85 90 95

Gly Lys Val Trp Gln Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn
100 105 110

Leu Glu Pro Asp Met Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu
115 120 125

Glu Ala Thr Gln Tyr Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu
130 135 140

Phe Thr Val Gln Ala Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp
145 150 155 160

Phe Pro Asp Ser Lys Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp
165 170 175

Gly Asp His Leu Ala Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe
180 185 190

Gly Phe Phe Arg Leu Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro
195 200 205

Asn Gly Glu Gln Arg Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu
210 215 220

Leu Val Asp Thr Leu Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His
225 230 235 240

Asp Gly Ile Ser Leu Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu
245 250 255

Thr Ala Gly Arg Thr Val Gln Leu Val Lys Asn Leu Phe Leu Ser Ser
260 265 270

Glu Arg Ser Tyr Trp Arg Lys Ala Asn Glu Ala Tyr Met Ala Leu Ile
275 280 285

Met Asp Ala Arg Tyr Ser Lys Asp Arg Ile Leu Glu Leu Tyr Met Asn
290 295 300

Glu Val Tyr Leu Gly Gln Ser Gly Asp Asn Glu Ile Arg Gly Phe Pro
305 310 315 320

Leu Ala Ser Leu Tyr Tyr Phe Gly Arg Pro Val Glu Glu Leu Ser Leu
325 330 335

Asp Gln Gln Ala Leu Leu Val Gly Met Val Lys Gly Ala Ser Ile Tyr
340 345 350

Asn Pro Trp Arg Asn Pro Lys Leu Ala Leu Glu Arg Arg Asn Leu Val
355 360 365

Leu Arg Leu Leu Gln Gln Gln Gln Ile Ile Asp Gln Glu Leu Tyr Asp
370 375 380

Met Leu Ser Ala Arg Pro Leu Gly Val Gln Pro Arg Gly Gly Val Ile
385 390 395 400

Ser Pro Gln Pro Ala Phe Met Gln Leu Val Arg Gln Glu Leu Gln Ala
405 410 415

Lys L u Gly Asp Lys Val Lys Asp Leu Ser Gly Val Lys Ile Phe Thr
420 425 430

Thr Phe Asp Ser Val Ala Gln Asp Ala Ala Glu Lys Ala Ala Val Glu
435 440 445

Gly Il Pro Ala Leu Lys Lys Gln Arg Lys Leu Ser Asp Leu Glu Thr
450 455 460

Ala Ile Val Val Val Asp Arg Phe Ser Gly Glu Val Arg Ala Met Val
465 470 475 480

Gly Gly Ser Glu Pro Gln Phe Ala Gly Tyr Asn Arg Ala Met Gln Ala
485 490 495

Arg Arg Ser Ile Gly Ser Leu Ala Lys Pro Ala Thr Tyr Leu Thr Ala
500 505 510

Leu Ser Gln Pro Lys Ile Tyr Arg Leu Asn Thr Trp Ile Ala Asp Ala
515 520 525

Pro Ile Ala Leu Arg Gln Pro Asn Gly Gln Val Trp Ser Pro Gln Asn
530 535 540

Asp Asp Arg Arg Tyr Ser Glu Ser Gly Arg Val Met Leu Val Asp Ala
545 550 555 560

Leu Thr Arg Ser Met Asn Val Pro Thr Val Asn Leu Gly Met Ala Leu
565 570 575

Gly Leu Pro Ala Val Thr Glu Thr Trp Ile Lys Leu Gly Val Pro Lys
580 585 590

Asp Gln Leu His Pro Val Pro Ala Met Leu Leu Gly Ala Leu Asn Leu
595 600 605

Thr Pro Ile Glu Val Ala Gln Ala Phe Gln Thr Ile Ala Ser Gly Gly
610 615 620

Asn Arg Ala Pro Leu Ser Ala Leu Arg Ser Val Ile Ala Glu Asp Gly
625 630 635 640

Lys Val Leu Tyr Gln Ser Phe Pro Gln Ala Glu Arg Ala Val Pro Ala
645 650 655

Gln Ala Ala Tyr Leu Thr Leu Trp Thr Met Gln Gln Val Val Gln Arg
660 665 670

Gly Thr Gly Arg Gln Leu Gly Ala Lys Tyr Pro Asn Leu His Leu Ala
675 680 685

Gly Lys Thr Gly Thr Thr Asn Asn Asn Val Asp Thr Trp Phe Ala Gly
690 695 700

Ile Asp Gly Ser Thr Val Thr Ile Thr Trp Val Gly Arg Asp Asn Asn
705 710 715 720

Gln Pro Thr Lys Leu Tyr Gly Ala Ser Gly Ala Met Ser Ile Tyr Gln
725 730 735

Arg Tyr Leu Ala Asn Gln Thr Pro Thr Pro Leu Asn Leu Val Pro Pro
740 745 750

Glu Asp Ile Ala Asp Met Gly Val Asp Tyr Asp Gly Asn Phe Val Cys
755 760 765

Ser Gly Gly Met Arg Ile Leu Pro Val Trp Thr Ser Asp Pro Gln Ser
770 775 780

Leu Cys Gln G Ser Glu Met Gln Gln Gln Pro Ser Gly Asn Pro Phe
785 790 795 800

Asp Gln Ser Ser Gln Pro Gln Gln Gln Pro Gln Gln Gln Pro Ala Gln
805 810 815

Gln Glu Gln Lys Asp Ser Asp Gly Val Ala Gly Trp Il Lys Asp M t
820 825 830

Phe Gly Ser Asn
835

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 850 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC0571 PBP 1A QQAA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Lys Phe Val Lys Tyr Phe Leu Ile Leu Ala Val Cys Cys Ile Leu
1 5 10 15

Leu Gly Ala Gly Ser Ile Tyr Gly Leu Tyr Arg Tyr Ile Glu Pro Gln
20 25 30

Leu Pro Asp Val Ala Thr Leu Lys Asp Val Arg Leu Gln Ile Pro Met
35 40 45

Gln Ile Tyr Ser Ala Asp Gly Glu Leu Ile Ala Gln Tyr Gly Glu Lys
50 55 60

Arg Arg Ile Pro Val Thr Leu Asp Gln Ile Pro Pro Glu Met Val Lys
65 70 75 80

Ala Phe Ile Ala Thr Glu Asp Ser Arg Phe Tyr Glu His His Gly Val
85 90 95

Asp Pro Val Gly Ile Phe Arg Ala Ala Ser Val Ala Leu Phe Ser Gly
100 105 110

His Ala Ser Gln Gly Ala Ser Thr Ile Thr Ala Ala Leu Ala Arg Asn
115 120 125

Phe Phe Leu Ser Pro Glu Arg Thr Leu Met Arg Lys Ile Lys Glu Val
130 135 140

Phe Leu Ala Ile Arg Ile Glu Gln Leu Leu Thr Lys Asp Glu Ile Leu
145 150 155 160

Glu Leu Tyr Leu Asn Lys Ile Tyr Leu Gly Tyr Arg Ala Tyr Gly Val
165 170 175

Gly Ala Ala Ala Gln Val Tyr Phe Gly Lys Thr Val Asp Gln Leu Thr
180 185 190

Leu Asn Glu Met Ala Val Ile Ala Gly Leu Pro Lys Ala Pro Ser Thr
195 200 205

Phe Asn Pro L u Tyr Ser Met Asp Arg Ala Val Ala Arg Arg Asn Val
210 215 220

Val L u Ser Arg Met Leu Asp Glu Gly Tyr Il Thr Gln Gln Gln Phe
225 230 235 240

Asp Gln Thr Arg Thr Glu Ala Ile Asn Ala Asn Tyr His Ala Pro Glu
245 250 255

Ile Ala Phe Ser Ala Pro Tyr Leu Ser Glu Met Val Arg Gln Glu Met
260 265 270

Tyr Asn Arg Tyr Gly Glu Ser Ala Tyr Glu Asp Gly Tyr Arg Ile Tyr
275 280 285

Thr Thr Ile Thr Arg Lys Val Gln Gln Ala Ala Gln Gln Ala Val Arg
290 295 300

Asn Asn Val Leu Asp Tyr Asp Met Arg His Gly Tyr Arg Gly Pro Ala
305 310 315 320

Asn Val Leu Trp Lys Val Gly Glu Ser Ala Trp Asp Asn Asn Lys Ile
325 330 335

Thr Asp Thr Leu Lys Ala Leu Pro Thr Tyr Gly Pro Leu Leu Pro Ala
340 345 350

Ala Val Thr Ser Ala Asn Pro Gln Gln Ala Thr Ala Met Leu Ala Asp
355 360 365

Gly Ser Thr Val Ala Leu Ser Met Glu Gly Val Arg Trp Ala Arg Pro
370 375 380

Tyr Arg Ser Asp Thr Gln Gln Gly Pro Thr Pro Arg Lys Val Thr Asp
385 390 395 400

Val Leu Gln Thr Gly Gln Gln Ile Trp Val Arg Gln Val Gly Asp Ala
405 410 415

Trp Trp Leu Ala Gln Val Pro Glu Val Asn Ser Ala Leu Val Ser Ile
420 425 430

Asn Pro Gln Asn Gly Ala Val Met Ala Leu Val Gly Gly Phe Asp Phe
435 440 445

Asn Gln Ser Lys Phe Asn Arg Ala Thr Gln Ala Leu Arg Gln Val Gly
450 455 460

Ser Asn Ile Lys Pro Phe Leu Tyr Thr Ala Ala Met Asp Lys Gly Leu
465 470 475 480

Thr Leu Ala Ser Met Leu Asn Asp Val Pro Ile Ser Arg Trp Asp Ala
485 490 495

Ser Ala Gly Ser Asp Trp Gln Pro Lys Asn Ser Pro Pro Gln Tyr Ala
500 505 510

Gly Pro Ile Arg Leu Arg Gln Gly Leu Gly Gln Ser Lys Asn Val Val
515 520 525

Met Val Arg Ala Met Arg Ala Met Gly Val Asp Tyr Ala Ala Glu Tyr
530 535 540

Leu Gln Arg Phe Gly Phe Pro Ala Gln Asn Ile Val His Thr Glu Ser
545 550 555 560

Leu Ala Leu Gly Ser Ala Ser Phe Thr Pro Met Gln Val Ala Arg Gly
565 570 575

Tyr Ala Val Met Ala Asn Gly Gly Phe Leu Val Asp Pro Trp Phe Il
580 585 590

Ser Lys Ile Glu Asn Asp Gln Gly Gly Val Ile Phe Glu Ala Lys Pro
595 600 605

Lys Val Ala Cys Pro Glu Cys Asp Ile Pro Val Ile Tyr Gly Asp Thr
610 615 620

Gln Lys Ser Asn Val Leu Glu Asn Asn Asp Val Glu Asp Val Ala Ile
625 630 635 640

Ser Arg Glu Gln Gln Asn Val Ser Val Pro Met Pro Gln Leu Glu Gln
645 650 655

Ala Asn Gln Ala Leu Val Ala Lys Thr Gly Ala Gln Glu Tyr Ala Pro
660 665 670

His Val Ile Asn Thr Pro Leu Ala Phe Leu Ile Lys Ser Ala Leu Asn
675 680 685

Thr Asn Ile Phe Gly Glu Pro Gly Trp Gln Gly Thr Gly Trp Arg Ala
690 695 700

Gly Arg Asp Leu Gln Arg Arg Asp Ile Gly Gly Lys Thr Gly Thr Thr
705 710 715 720

Asn Ser Ser Lys Asp Ala Trp Phe Ser Gly Tyr Gly Pro Gly Val Val
725 730 735

Thr Ser Val Trp Ile Gly Phe Asp Asp His Arg Arg Asn Leu Gly His
740 745 750

Thr Thr Ala Ser Gly Ala Ile Lys Asp Gln Ile Ser Gly Tyr Glu Gly
755 760 765

Gly Ala Lys Ser Ala Gln Pro Ala Trp Asp Ala Tyr Met Lys Ala Val
770 775 780

Leu Glu Gly Val Pro Glu Gln Pro Leu Thr Pro Pro Pro Gly Ile Val
785 790 795 800

Thr Val Asn Ile Asp Arg Ser Thr Gly Gln Leu Ala Asn Gly Gly Asn
805 810 815

Ser Arg Glu Glu Tyr Phe Ile Glu Gly Thr Gln Pro Thr Gln Gln Ala
820 825 830

Val His Glu Val Gly Thr Thr Ile Ile Asp Asn Gly Glu Ala Gln Glu
835 840 845

Leu Phe
850

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 553 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC 0592 truncated PBP 1B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
1 5 10 15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
20 25 30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
35 40 45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
50 55 60

Trp Leu Trp Leu Leu Leu Lys Leu Ala Ile Val Phe Ala Val Leu Ile
65 70 75 80

Ala Ile Tyr Gly Val Tyr Leu Asp Gln Lys Ile Arg Ser Arg Ile Asp
85 90 95

Gly Lys Val Trp Gln Leu Ala Ala Ala Val Tyr Gly Arg Met Val Asn
100 105 110

Leu Glu Pro Asp Met Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu
115 120 125

Glu Ala Thr Gln Tyr Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu
130 135 140

Phe Thr Val Gln Ala Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp
145 150 155 160

Phe Pro Asp Ser Lys Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp
165 170 175

Gly Asp His Leu Ala Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe
180 185 190

Gly Phe Phe Arg Leu Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro
195 200 205

Asn Gly Glu Gln Arg Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu
210 215 220

Leu Val Asp Thr Leu Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His
225 230 235 240

Asp Gly Ile Ser Leu Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu
245 250 255

Thr Ala Gly Arg Thr Val Gln Gly Ala Ser Thr Leu Thr Gln Gln Leu
260 265 270

Val Lys Asn Leu Phe Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala
275 280 285

Asn Glu Ala Tyr Met Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp
290 295 300

Arg Ile Leu Glu Leu Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly
305 310 315 320

Asp Asn Glu Ile Arg Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly
325 330 335

Arg Pro Val Glu Glu Leu Ser Leu Asp Gln Gln Ala Leu Leu Val Gly
340 345 350

```
Met Val Lys Gly Ala S r Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu
        355                 360                 365

Ala Leu Glu Arg Arg Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Gln
    370                 375                 380

Ile Ile Asp Gln Glu Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly
385                 390                 395                 400

Val Gln Pro Arg Gly Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln
                405                 410                 415

Leu Val Arg Gln Glu Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp
            420                 425                 430

Leu Ser Gly Val Lys Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp
        435                 440                 445

Ala Ala Glu Lys Ala Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln
    450                 455                 460

Arg Lys Leu Ser Asp Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe
465                 470                 475                 480

Ser Gly Glu Val Arg Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala
                485                 490                 495

Gly Tyr Asn Arg Ala Met Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala
            500                 505                 510

Lys Pro Ala Thr Tyr Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg
        515                 520                 525

Leu Asn Thr Trp Ile Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn
    530                 535                 540

Gly Gln Val Trp Ser Pro Gln Asn Asp
545                 550
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC 0593 truncated soluble PBP 1B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Met Ala Gly Asn Asp Arg Glu Pro Ile Gly Arg Lys Gly Lys Pro Thr
1               5                   10                  15

Arg Pro Val Lys Gln Lys Val Ser Arg Arg Arg Tyr Glu Asp Asp Asp
            20                  25                  30

Asp Tyr Asp Asp Tyr Asp Asp Tyr Glu Asp Glu Glu Pro Met Pro Arg
        35                  40                  45

Lys Gly Lys Gly Lys Gly Lys Gly Arg Lys Pro Arg Gly Lys Arg Gly
    50                  55                  60
```

Ser Ile Asp Gln Lys Ile Arg Ser Arg Ile Asp Gly Lys Val Trp Gln
65 70 75 80

Leu Ala Ala Ala Val Tyr Gly Arg M t Val Asn Leu Glu Pro Asp Met
85 90 95

Thr Ile Ser Lys Asn Glu Met Val Lys Leu Leu Glu Ala Thr Gln Tyr
100 105 110

Arg Gln Val Ser Lys Met Thr Arg Pro Gly Glu Phe Thr Val Gln Ala
115 120 125

Asn Ser Ile Glu Met Ile Arg Arg Pro Phe Asp Phe Pro Asp Ser Lys
130 135 140

Glu Gly Gln Val Arg Ala Arg Leu Thr Phe Asp Gly Asp His Leu Ala
145 150 155 160

Thr Ile Val Asn Met Glu Asn Asn Arg Gln Phe Gly Phe Phe Arg Leu
165 170 175

Asp Pro Arg Leu Ile Thr Met Ile Ser Ser Pro Asn Gly Glu Gln Arg
180 185 190

Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu Leu Val Asp Thr Leu
195 200 205

Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His Asp Gly Ile Ser Leu
210 215 220

Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu Thr Ala Gly Arg Thr
225 230 235 240

Val Gln Gly Ala Ser Thr Leu Thr Gln Gln Leu Val Lys Asn Leu Phe
245 250 255

Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala Asp Glu Ala Tyr Met
260 265 270

Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp Arg Ile Leu Glu Leu
275 280 285

Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly Asp Asn Glu Ile Arg
290 295 300

Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly Arg Pro Val Glu Glu
305 310 315 320

Leu Ser Leu Asp Gln Gln Ala Leu Leu Val Gly Met Val Lys Gly Ala
325 330 335

Ser Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu Ala Leu Glu Arg Arg
340 345 350

Asn Leu Val Leu Arg Leu Leu Gln Gln Gln Ile Ile Asp Gln Glu
355 360 365

Leu Tyr Asp Met Leu Ser Ala Arg Pro Leu Gly Val Gln Pro Arg Gly
370 375 380

Gly Val Ile Ser Pro Gln Pro Ala Phe Met Gln Leu Val Arg Gln Glu
385 390 395 400

Leu Gln Ala Lys Leu Gly Asp Lys Val Lys Asp Leu Ser Gly Val Lys
405 410 415

Ile Phe Thr Thr Phe Asp Ser Val Ala Gln Asp Ala Ala Glu Lys Ala
420 425 430

Ala Val Glu Gly Ile Pro Ala Leu Lys Lys Gln Arg Lys Leu Ser Asp
435 440 445

Leu Glu Thr Ala Ile Val Val Val Asp Arg Phe Ser Gly Glu Val Arg
450 455 460

Ala Met Val Gly Gly Ser Glu Pro Gln Phe Ala Gly Tyr Asn Arg Ala
465 470 475 480

Met Gln Ala Arg Arg Ser Ile Gly Ser Leu Ala Lys Pro Ala Thr Tyr
485 490 495

Leu Thr Ala Leu Ser Gln Pro Lys Ile Tyr Arg Leu Asn Thr Trp Ile
500 505 510

Ala Asp Ala Pro Ile Ala Leu Arg Gln Pro Asn Gly Gln Val Trp Ser
515 520 525

Pro Gln Asn Asp
530

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vii) IMMEDIATE SOURCE:
(B) CLONE: pARC 0392

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Gly Glu Gln Arg Leu Phe Val Pro Arg Ser Gly Phe Pro Asp Leu Leu
1 5 10 15

Val Asp Thr Leu Leu Ala Thr Glu Asp Arg His Phe Tyr Glu His Asp
20 25 30

Gly Ile Ser Leu Tyr Ser Ile Gly Arg Ala Val Leu Ala Asn Leu Thr
35 40 45

Ala Gly Arg Thr Val Gln Gly Ala Ser Thr Leu Thr Gln Gln Leu Val
50 55 60

Lys Asn Leu Phe Leu Ser Ser Glu Arg Ser Tyr Trp Arg Lys Ala Asn
65 70 75 80

Glu Ala Tyr Met Ala Leu Ile Met Asp Ala Arg Tyr Ser Lys Asp Arg
85 90 95

Ile Leu Glu Leu Tyr Met Asn Glu Val Tyr Leu Gly Gln Ser Gly Asp
100 105 110

Asn Glu Ile Arg Gly Phe Pro Leu Ala Ser Leu Tyr Tyr Phe Gly Arg
115 120 125

Pro Val Glu Glu L u Ser Leu Asp Gln Gln Ala Leu Leu Val Gly Met
130 135 140

Val Lys Gly Ala Ser Ile Tyr Asn Pro Trp Arg Asn Pro Lys Leu
145 150 155

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 18

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40666

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international applicati n	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1992)

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 10.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40667

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 27, line 2.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40665

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 29, line 19.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40661

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

Th indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 29, line 23.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40662

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 29, line 27.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40663

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 34, line 3.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40668

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 36, line 7.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40669

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 36, line 15.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40670

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 37, line 11.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40659

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 38, line 23.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40664

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 40, line 13.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
Aberdeen AB2 1RY
Scotland, UK

Date of deposit	Accession Number
28 June 1994	NCIMB 40660

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions *mutatis mutandis* for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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CLAIMS

1. A polypeptide which is a water-soluble active derivative of a bacterial bifunctional penicillin binding protein, said penicillin binding protein being bound to the cell membrane when expressed in a bacterial cell and being capable of exhibiting both transglycosylase and transpeptidase activities and said derivative lacking a membrane anchoring sequence but retaining the capability to exhibit one or both of said enzymic activities.

2. A polypeptide according to claim 1 which amino acid sequence is identical to, or substantially similar to, SEQ ID NO: 2, 4, 6, 12 or 13 in the Sequence Listing.

3. A polypeptide which is a transglycosylase deficient derivative of a bacterial bifunctional penicillin binding protein, said penicillin binding protein being bound to the cell membrane when expressed in a bacterial cell and being capable of exhibiting both transglycosylase and transpeptidase activities and said derivative lacking the capability to exhibit transglycosylase activity but retaining the capability to exhibit transpeptidase activity.

4. A polypeptide according to claim 3 wherein the said derivative is lacking transglycosylase activity because of a mutation or deletion in the second conserved region of the gene coding for said polypeptide.

5. A polypeptide according to claim 3 which amino acid sequence is identical to, or substantially similar to, SEQ ID NO: 7, 8, 9, or 10 in the Sequence Listing.

6. A polypeptide according to claim 1 or 3 wherein the bacterial cell is an *Escherichia coli* cell or a *Streptococcus pneumoniae* cell.

7. A polypeptide comprising (a) a first polypeptide according to claim 1 or 3; and (b) an additional polypeptide which allows binding to an affinity matrix; there being a cleavage site between said polypeptides.

8. A polypeptide according to claim 7 wherein the additional polypeptide is glutathione-S-transferase or a polypeptide substantially similar to glutathione-S-transferase.

9. A polypeptide according to claim 7 wherein the additional polypeptide is a polypeptide rich in histidine residues.

10. An isolated and purified DNA molecule which has a nucleotide sequence coding for a polypeptide according to claim 1, 3 or 7.

11. A DNA molecule according to claim 10, which nucleotide sequence is identical to, or substantially similar to, SEQ ID NO: 1, 3 or 5 in the Sequence Listing.

12. A replicable expression vector which carries and is capable of mediating the expression of a DNA molecule according to claim 10.

13. A vector according to claim 12 which is the vector
pARC0558 (NCIMB No. 40666),
pARC0559 (NCIMB No. 40667),
pARC0512 (NCIMB No. 40665),
pARC0438 (NCIMB No. 40661),
pARC0468 (NCIMB No. 40662),
pARC0469 (NCIMB No. 40663),

pARC0571 (NCIMB No. 40668),
pARC0593 (NCIMB No. 40670),
pARC0392 (NCIMB No. 40659),
pARC0499 (NCIMB No. 40664), or
pARC0400 (NCIMB No. 40660).

14. A cell harbouring a vector according to claim 12.

15. A process for production of a polypeptide which is a derivative of penicillin binding protein, comprising growing a cell according to claim 14 in or on a culture medium for expression of the polypeptide and optionally recovering the polypeptide.

16. A process for the production of a water soluble polypeptide according to claim 1 which comprises culturing *Escherichia coli* cells harbouring an expression vector wherein a DNA coding sequence for said polypeptide is under the control of an isopropyl thiogalactoside (IPTG) - inducible promoter, said culturing being carried out in the presence of a sub-optimal concentration of IPTG for induction of the said promoter and at a temperature in the range of 20 to 24°C.

17. A method of identifying an antibody capable of binding a bacterial bifunctional penicillin binding protein which includes the step of employing a polypeptide according to claim 1 or 3 in an antibody binding assay and selecting antibodies that bind to the polypeptide.

18. A method of assaying for compounds which bind to a penicillin binding protein, said method comprising (a) contacting a polypeptide according to claim 1, 3 or 7 with a compound to be investigated; and (b) detecting whether said compound binds to the penicillin binding protein.

19. A method of assaying for compounds which bind to a penicillin binding protein, said method comprising (a) culturing cells according to claim 14; (b) lysing the said cells and isolating the crude cell extract; (c) exposing the said cell extract to potential inhibitors of a penicillin binding protein; (d) introducing an agent, known to bind a penicillin binding protein, to the said cell extract; (e) removing the unbound fraction of said agent; and (f) assaying the presence of said agent remaining in the cell extract.

20. A method of assaying for compounds which bind to a penicillin binding protein, said method comprising (a) exposing a polypeptide according to claim 1, 3 or 7, immobilised on a solid support, to a potential inhibitor of a penicillin binding protein; (b) exposing an agent, known to bind a penicillin binding protein, to the immobilised polypeptide; (c) removing the unbound fraction of said agent; and (d) assaying the presence of said agent bound to the immobilised polypeptide.

21. A method of assaying for compounds which bind to a penicillin binding protein, said method comprising (a) exposing a polypeptide according to claim 1, 3 or 7 to a potential inhibitor of a penicillin binding protein; (b) exposing the said polypeptide to an agent, known to bind a penicillin binding protein, which agent is immobilised on a solid support; and (c) assaying the presence of polypeptide bound to the immobilised agent.

22. A method of assaying for compounds which bind to the transglycosylase domain of a penicillin binding protein, said method comprising (a) exposing the transglycosylase domain of a polypeptide according to claim 1 or 7, said polypeptide being immobilised on a solid support, to a potential inhibitor of the transglycosylase activity of a penicillin binding protein; (b) exposing

an agent, known to bind the transglycosylase domain of a penicillin binding protein, to the immobilised polypeptide; (c) removing the unbound fraction of said agent; and (d) assaying the presence of said agent bound to the immobilised polypeptide.

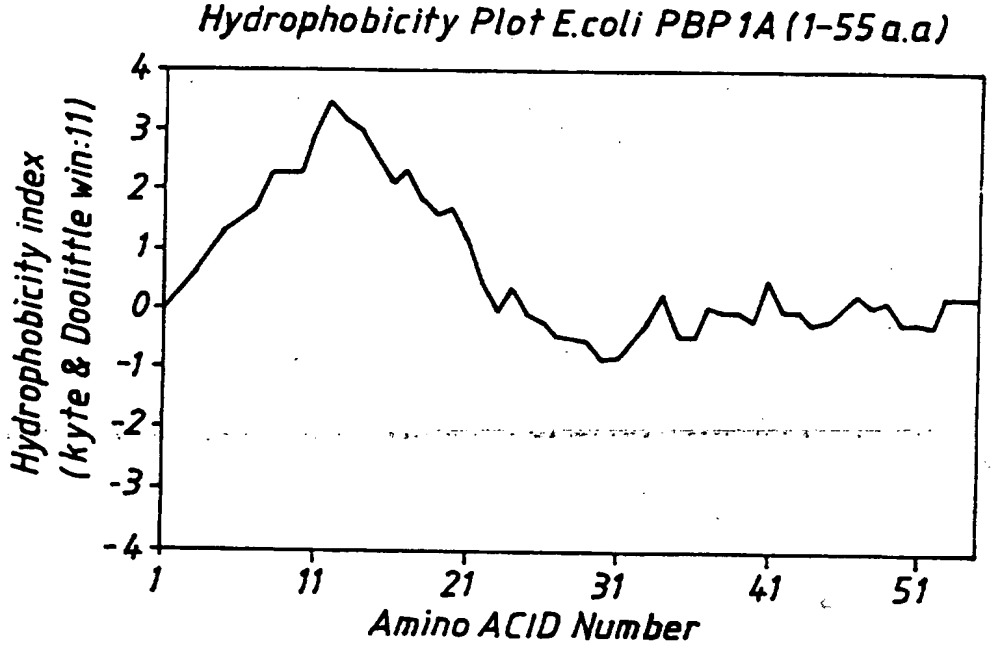
23. A method of assaying for compounds which bind to the transglycosylase domain of a penicillin binding protein, said method comprising (a) exposing the transglycosylase domain of a polypeptide according to claim 1 or 7 to a potential inhibitor of a penicillin binding protein; (b) exposing the said polypeptide to an agent, known to bind to the transglycosylase domain of a penicillin binding protein, which agent is immobilised on a solid support; and (c) assaying the presence of polypeptide bound to the immobilised agent.

24. A method according to any one of claims 19 to 23 wherein the agent known to bind a penicillin binding protein is a monoclonal antibody.

25. A method according to any one of claims 19 to 23 wherein the agent known to bind a penicillin binding protein is a labelled antibiotic compound.

26. A method of determining the protein structure of a penicillin binding protein, characterized in that a polypeptide according to claim 1 or 3 is utilized in X-ray crystallography.

Fig. 1


Hydrophobicity Plot E.coli PBP 1A (1-55 a.a)
Hydrophobicity index
(kyte & Doolittle win:11)
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3
2
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-3
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11
21
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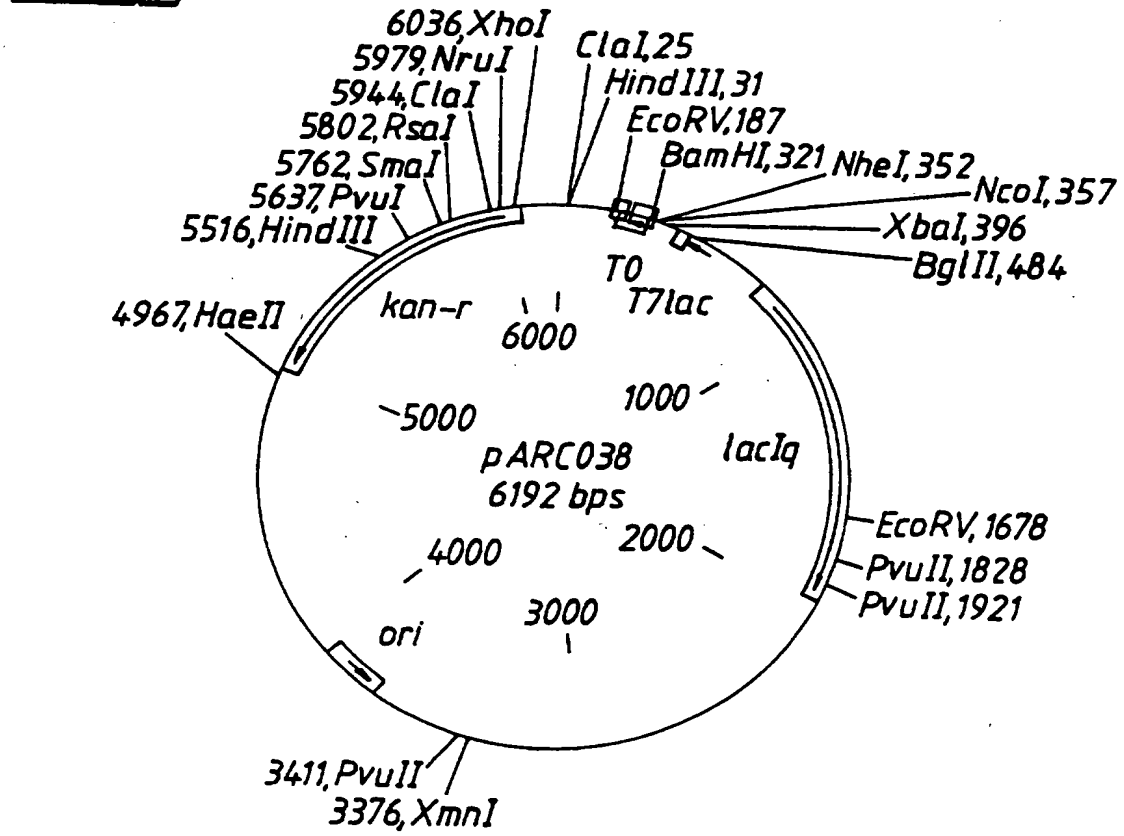
41
51
Amino ACID Number

Fig. 2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BamHI,321
NheI,352
NcoI,357
XbaI,396
BglII,484
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kan-r
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pARC038
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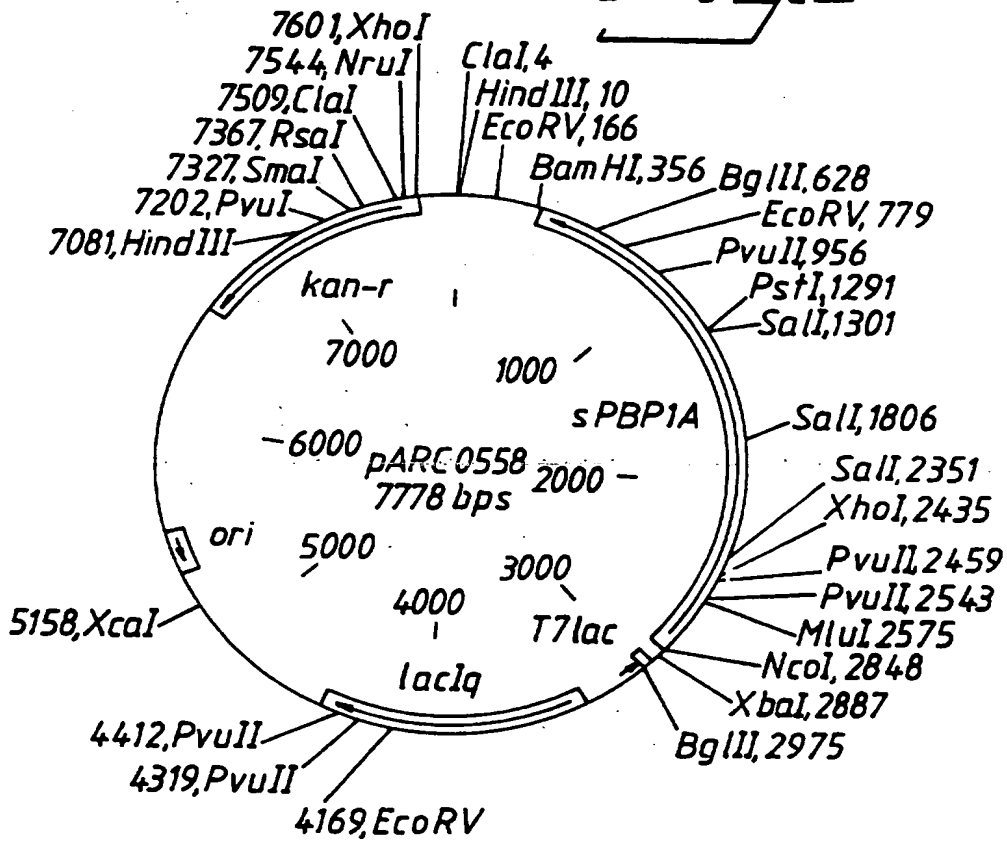
Fig. 3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7367, RsaI
7327, SmaI
7202, PvuI
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HindIII, 10
EcoRV, 166
BamHI, 356
BglII, 628
EcoRV, 779
PvuII, 956
PstI, 1291
SalI, 1301
SalI, 1806
SalI, 2351
XhoI, 2435
PvuII, 2459
PvuII, 2543
MluI, 2575
NcoI, 2848
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BglII, 2975
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sPBP1A
6000
pARC0558
7778 bps
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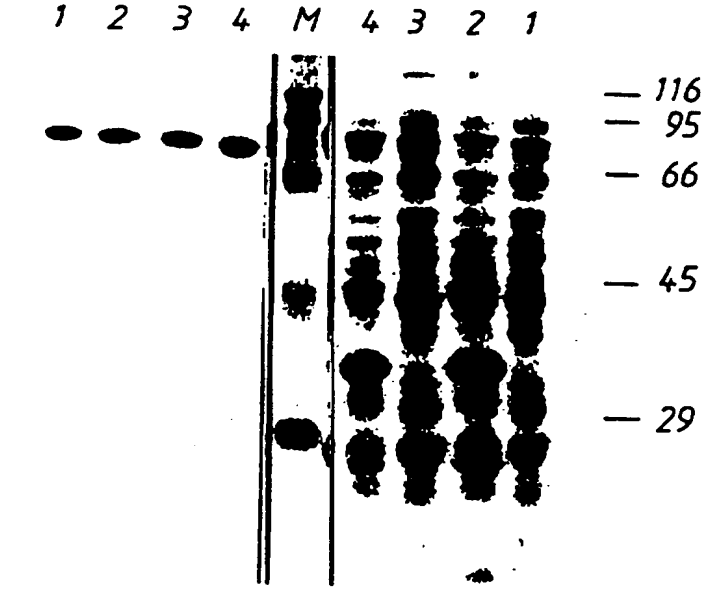
4319, PvuII
4169, EcoRV

Fig. 4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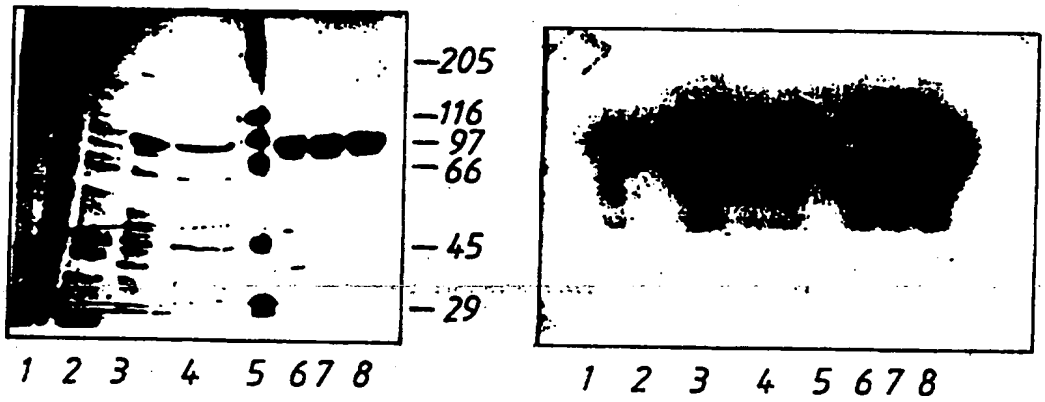
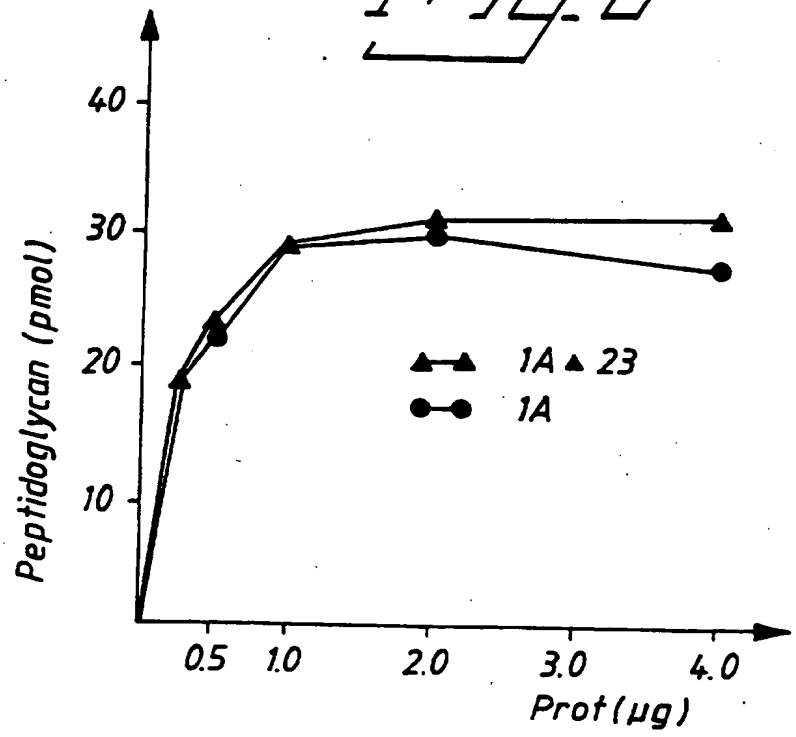
116
95
66
45
29
Panel A
Panel B

Fig. 5



Panel A Panel B

Fig. 6



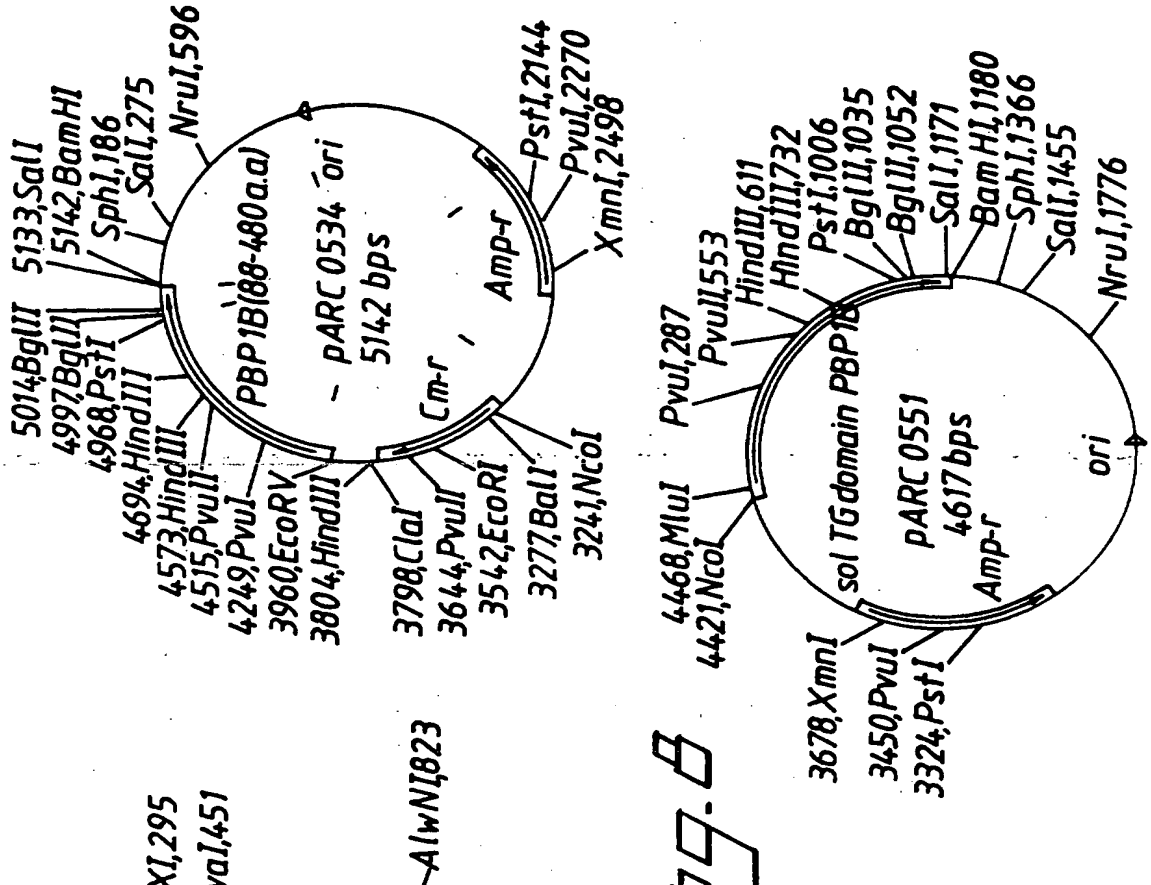
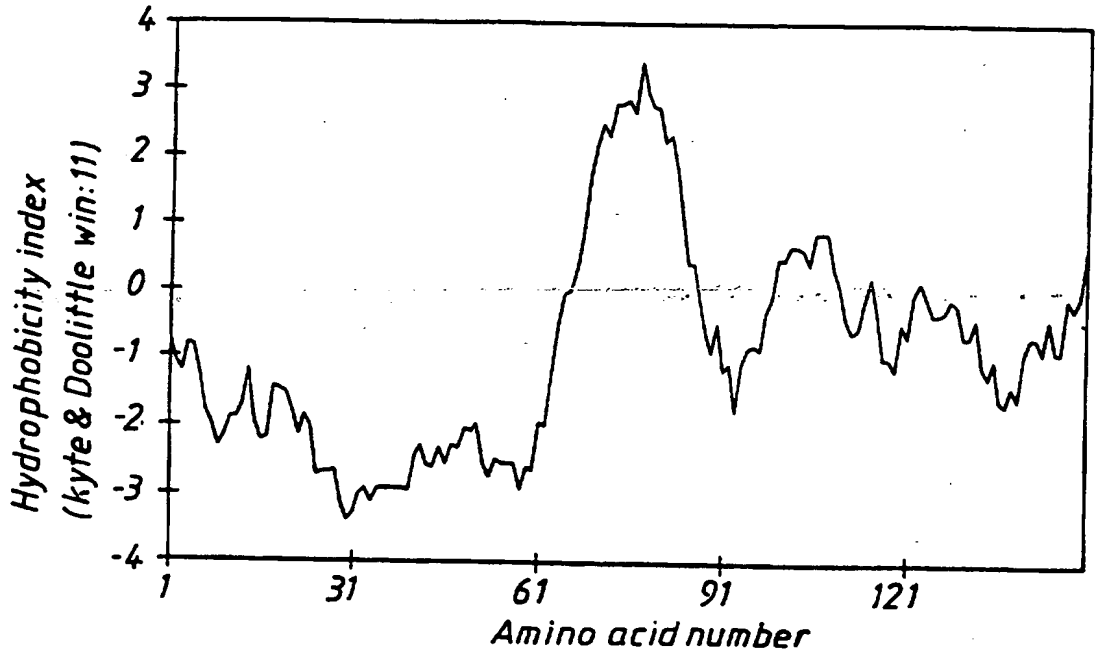


Fig. 8

NcoI - NruI fragment from pARC0547 cloned into NcoI -EcoRV sites of pARC0534.

Fig. 7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Hydrophobicity index
(kyte & Doolittle win:11)
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3
2
1
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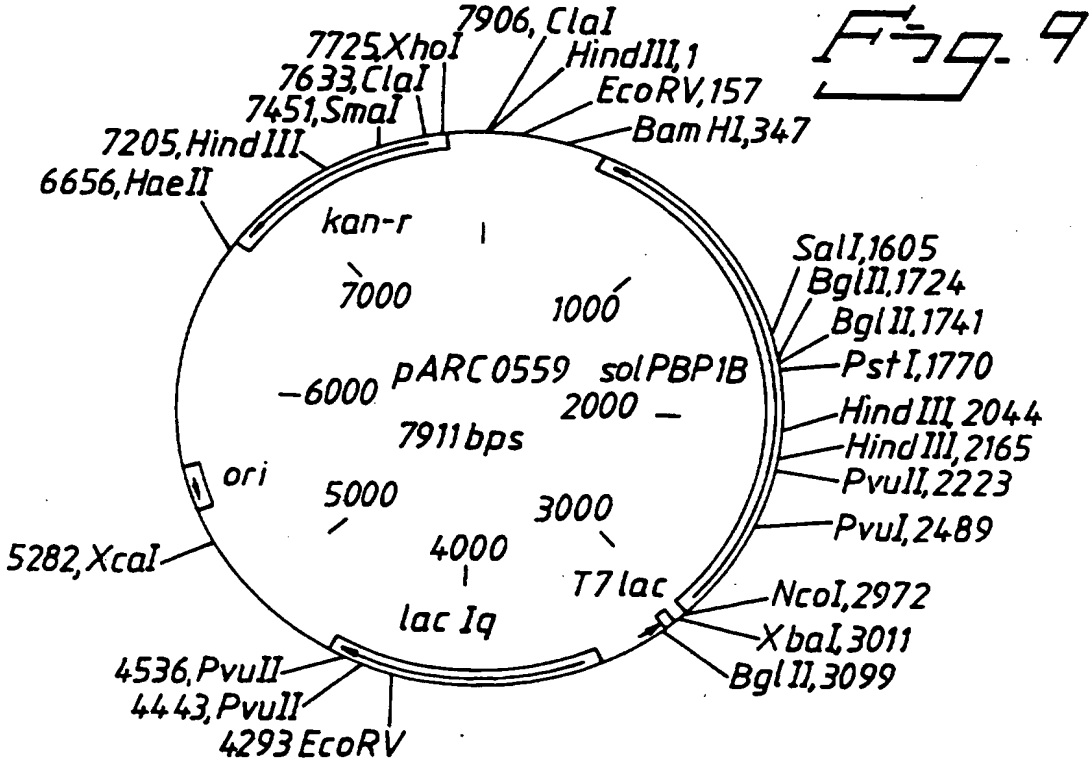
61
91
121
Amino acid number

Fig. 9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EcoRV,157
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BamHI,347
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kan-r
SalI,1605
BglII,1724
BglII,1741
PstI,1770
HindIII,2044
HindIII,2165
PvuII,2223
PvuI,2489
NcoI,2972
XbaI,3011
BglII,3099
4536,PvuII
4443,PvuII
4293 EcoRV
5282,XcaI
ori
7000
1000
6000
2000
5000
3000
4000
pARC0559 solPBP1B
7911 bps
T7 lac
lac Iq

Fig. 10

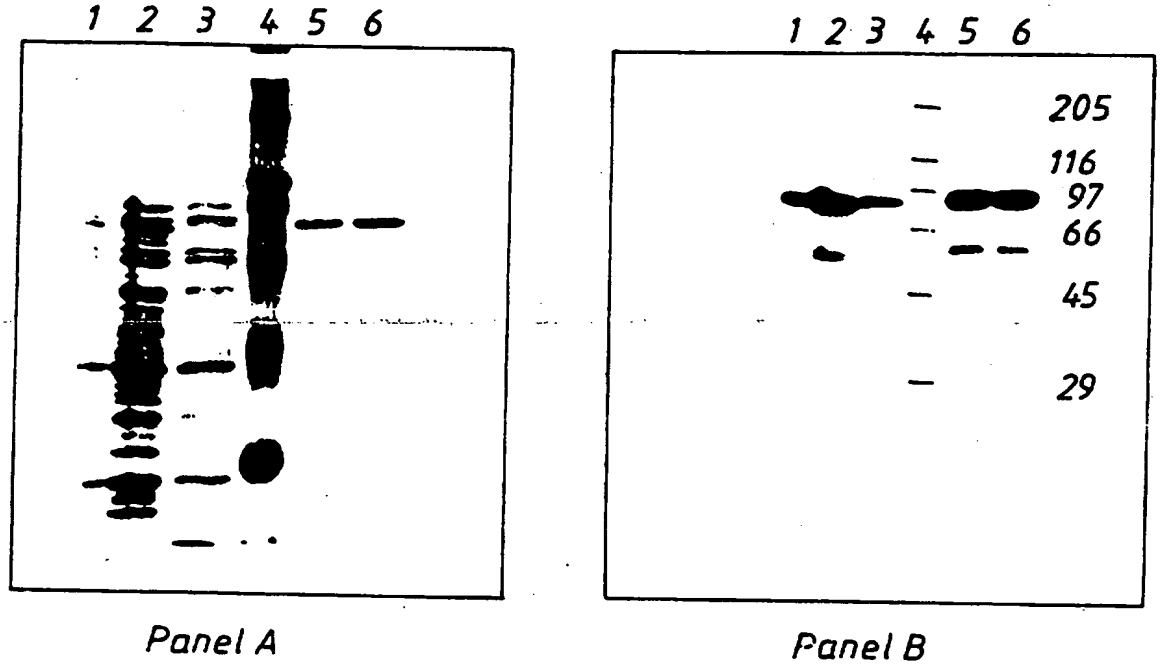

1 2 3 4 5 6
1 2 3 4 5 6
205
116
97
66
45
29
Panel A
Panel B

Fig. 11

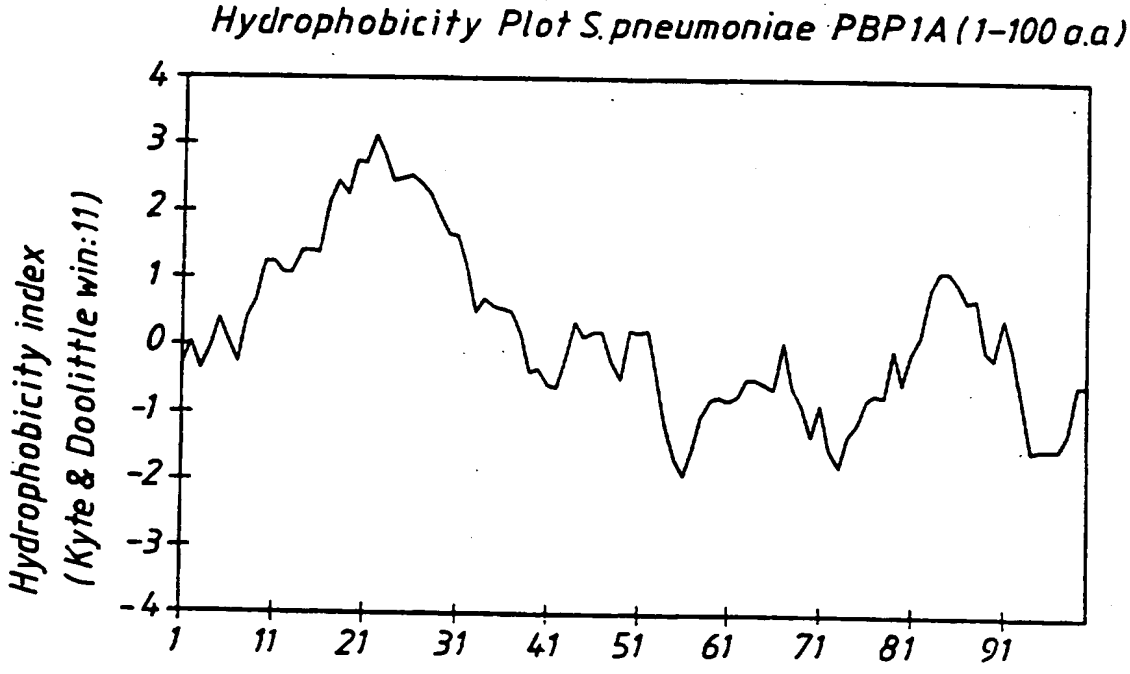

Hydrophobicity Plot S.pneumoniae PBP1A (1-100 a.a)
Hydrophobicity index
(Kyte & Doolittle win:11)
4
3
2
1
0
-1
-2
-3
-4
1
11
21
31
41
51
61
71
81
91

Fig. 12

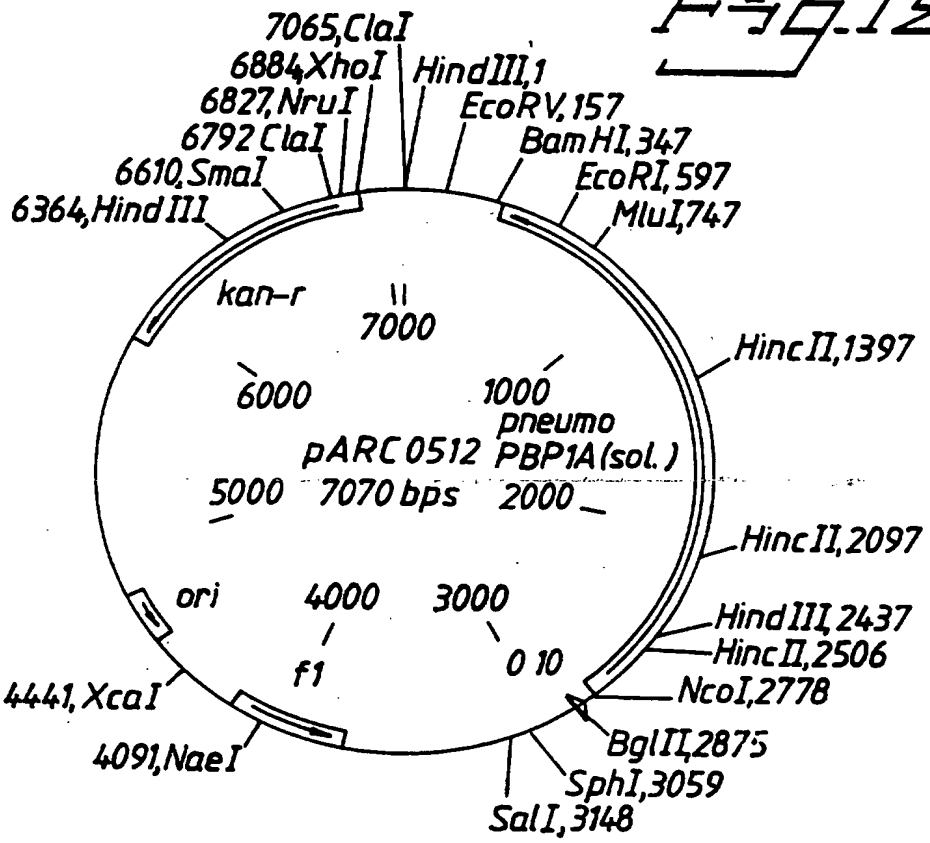
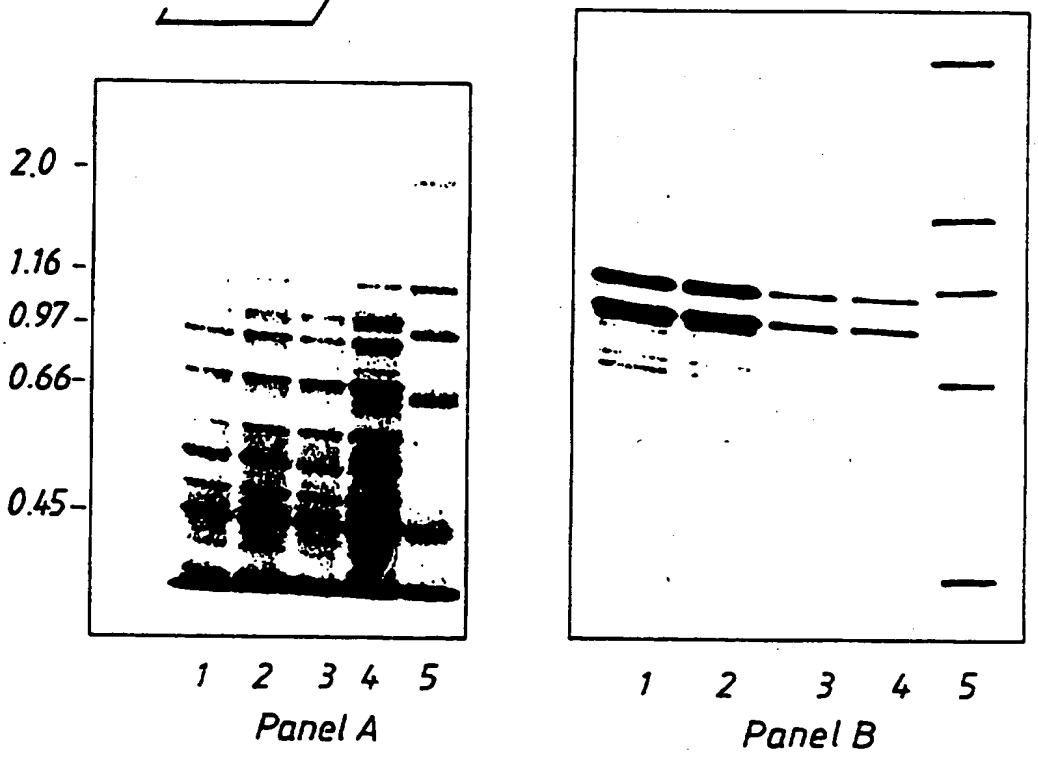

7065,ClaI
6884,XhoI
6827,NruI
6792 ClaI
6610,SmaI
6364,HindIII
HindIII,1
EcoRV,157
BamHI,347
EcoRI,597
MluI,747
kan-r
7000
6000
1000
pneumo
pARC 0512 PBP1A(sol.)
5000 7070 bps 2000
HincII,1397
HincII,2097
HindIII,2437
HincII,2506
NcoI,2778
BglII,2875
SphI,3059
SalI,3148
ori
4000
3000
f1
0 10
4441,XcaI
4091,NaeI

Fig. 13


2.0
1.16
0.97
0.66
0.45
1 2 3 4 5
Panel A
1 2 3 4 5
Panel B

```
E.1B 190  DPRLITMISSPNGEQR.LFVPRSGFPDLLVDTLLATEDRHFYEHDGISLYSIGRAVLANLTAGRTVQGASTLTQQLVKNLFLS...
S.1A  55  IYDNKNQLIADLGSERRVNAQANDIPTDLVKAIVSIEDHRFFDHRGIDTIRILGAFLRNLQSN.SLQGGSALTQQLIKLTYFSTST
E.1A  50  IYSADGELIAQYGEKRRIPVTLDQIPPEMVKAFIATEDSRFYEHHGVDPVGIFRAASVALFSGHASQGASTITQQLARNFFLS...
H.inf 50  IYTADGKLGGEVGEQRRIPVKLADVPQRLIDAFLATEDSRFYDHHGLDPIGIARALFVFYSNGGASQGASTITQQLARNFFLI...

                                  REGION 1                                  REGION 2

E.1B      SERSYWRKANEAYMALIMDARYSKDRILELYMNEVYLGQSGDNEIRGFPLASLYYFGRPVEELSLDQQALLVGMVKGASIYNPWR
S.1A      SDQTISRKAQEAWLAIQLEQKATKQEILTYYINKVYMS....NGNYGMQTAAQNYYGKDLNNLSLPQLALLAGMPQAPNQYDPYS
E.1A      PERTLMRKIKEVELAIRIEQLLTKDEILELYLNKIYLG....YRAYGVGAAAQVYFGKTVDQLTLNEMAVIAGLPKAPSTFNPLY
H.inf     SEKTIIKKFKEAVLAVEIENTLNKQEILELYLNKIFLG....YRSYGVAAAAQTYFGKSLNLLTLSEMAIIAGLPFAFSTMNALY

                                  REGION 3

E.1B      NPKLALERRNLVLRLLQQQQII    386
S.1A      HPEAAQDRRNLVLSEMKNQGYI    242
E.1A      SMDRAVARRNVVLSRMLDEGYI    235
H.inf     SLKRSEERRNVVLSRMLDEKYI    236
```

Fig 14

Fig. 15

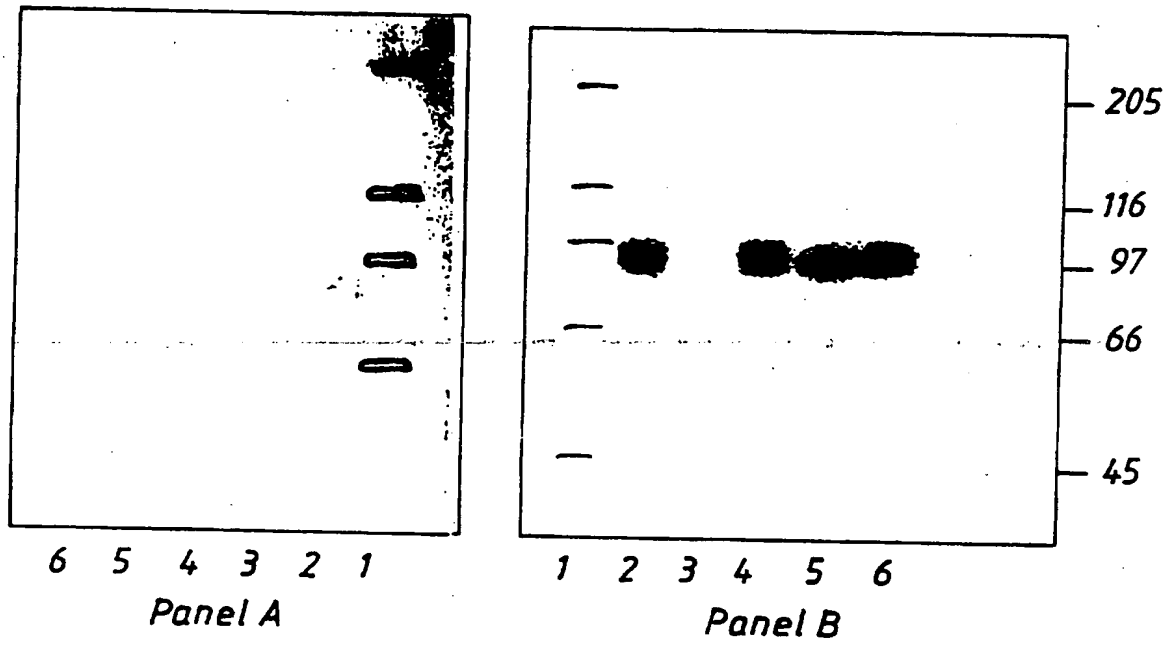

205
116
97
66
45
6 5 4 3 2 1
Panel A
1 2 3 4 5 6
Panel B

Fig. 16

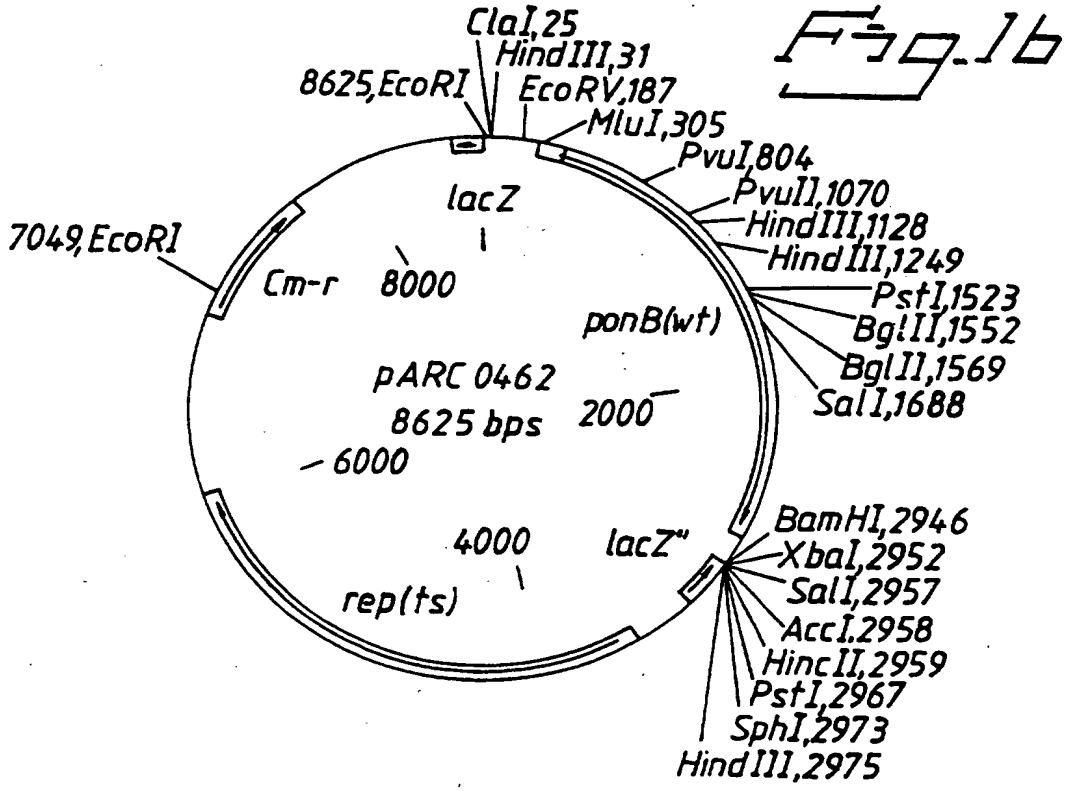

ClaI,25
HindIII,31
8625,EcoRI
EcoRV,187
MluI,305
PvuI,804
PvuII,1070
HindIII,1128
HindIII,1249
PstI,1523
BglII,1552
BglII,1569
SalI,1688
lacZ
7049,EcoRI
Cm-r
8000
ponB(wt)
pARC 0462
8625 bps
2000
6000
4000
lacZ"
rep(ts)
BamHI,2946
XbaI,2952
SalI,2957
AccI,2958
HincII,2959
PstI,2967
SphI,2973
HindIII,2975

Fig. 17

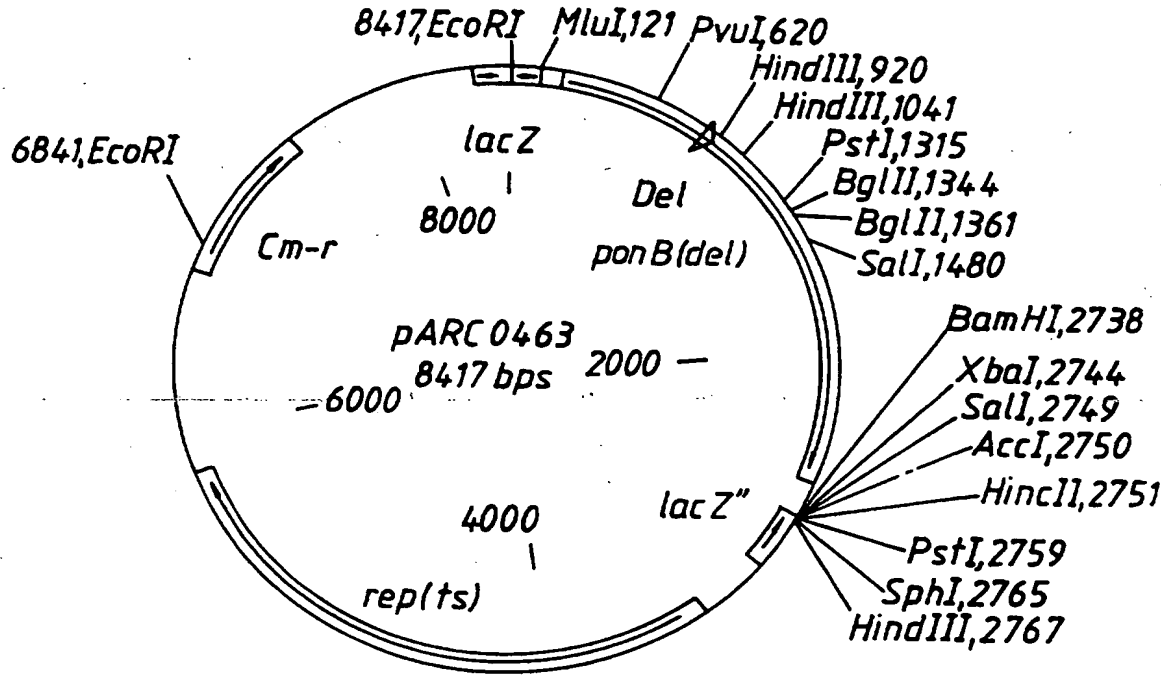

8417,EcoRI
MluI,121
PvuI,620
HindIII,920
HindIII,1041
PstI,1315
BglII,1344
BglII,1361
SalI,1480
6841,EcoRI
lacZ
8000
Del
Cm-r
ponB(del)
pARC 0463
8417 bps
2000
6000
BamHI,2738
XbaI,2744
SalI,2749
AccI,2750
HincII,2751
4000
lacZ"
PstI,2759
SphI,2765
HindIII,2767
rep(ts)

Fig. 18

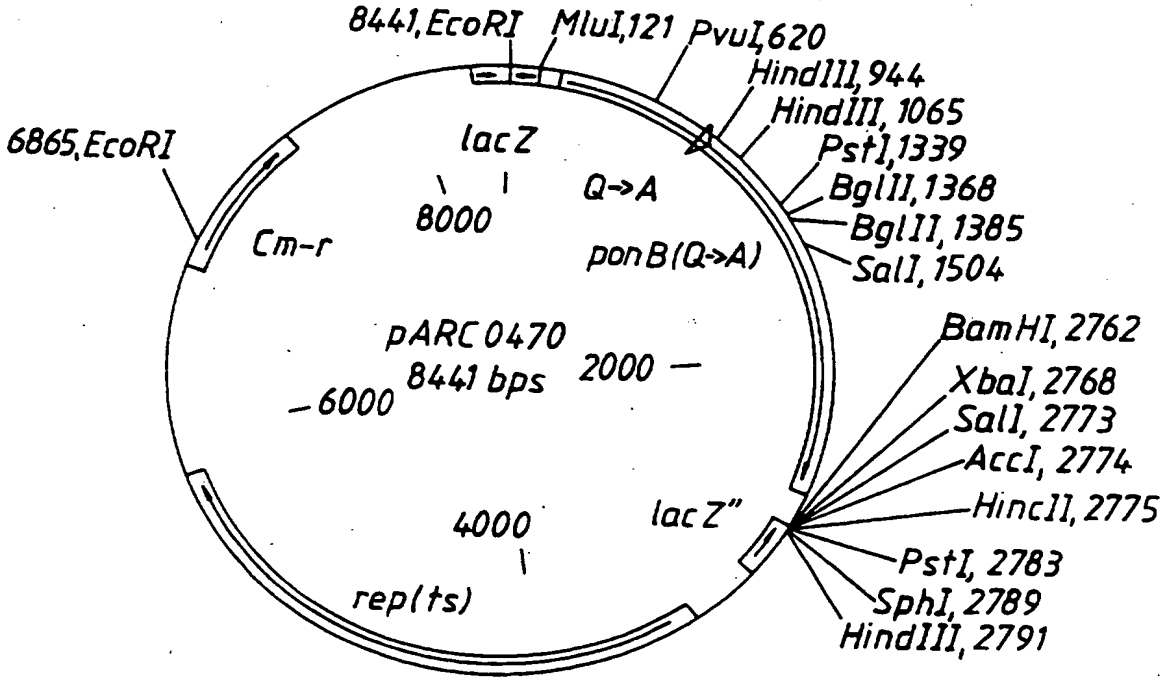

8441,EcoRI
MluI,121
PvuI,620
HindIII,944
HindIII, 1065
PstI,1339
BglII,1368
BglII, 1385
SalI, 1504
6865,EcoRI
lacZ
8000
Q->A
Cm-r
ponB(Q->A)
pARC 0470
8441 bps
2000
6000
BamHI, 2762
XbaI, 2768
SalI, 2773
AccI, 2774
HincII, 2775
4000
lacZ"
PstI, 2783
SphI, 2789
HindIII, 2791
rep(ts)

Fig. 19

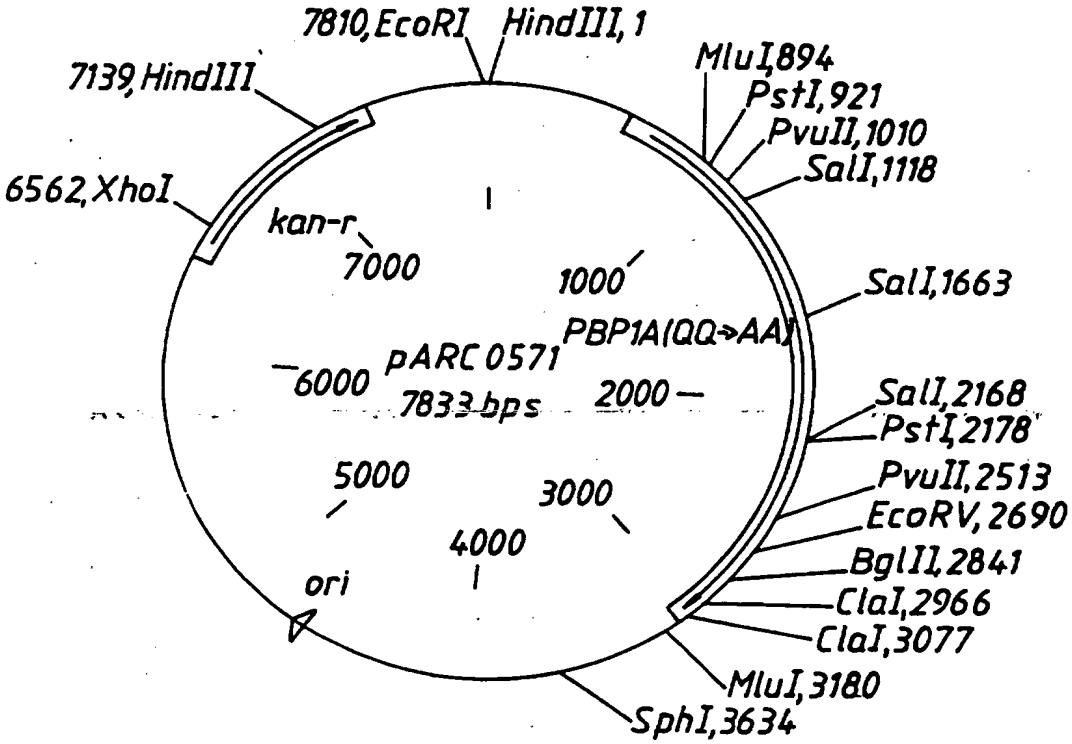

7810,EcoRI
HindIII, 1
MluI,894
PstI,921
PvuII,1010
SalI,1118
7139,HindIII
6562,XhoI
kan-r
7000
1000
SalI,1663
PBP1A(QQ→AA)
pARC 0571
6000
7833 bps
2000
SalI,2168
PstI,2178
5000
3000
PvuII,2513
EcoRV,2690
4000
BglII,2841
ori
ClaI,2966
ClaI,3077
MluI,3180
SphI,3634

Fig. 20

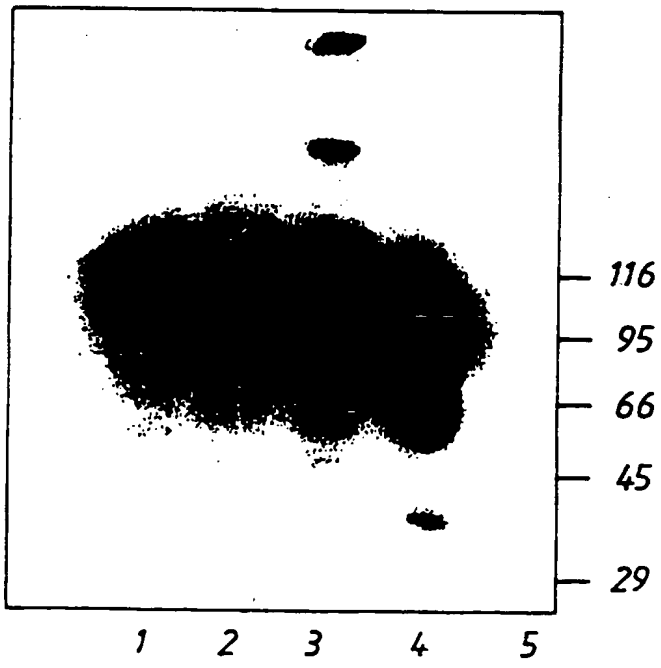

116
95
66
45
29
1
2
3
4
5

Fig. 21

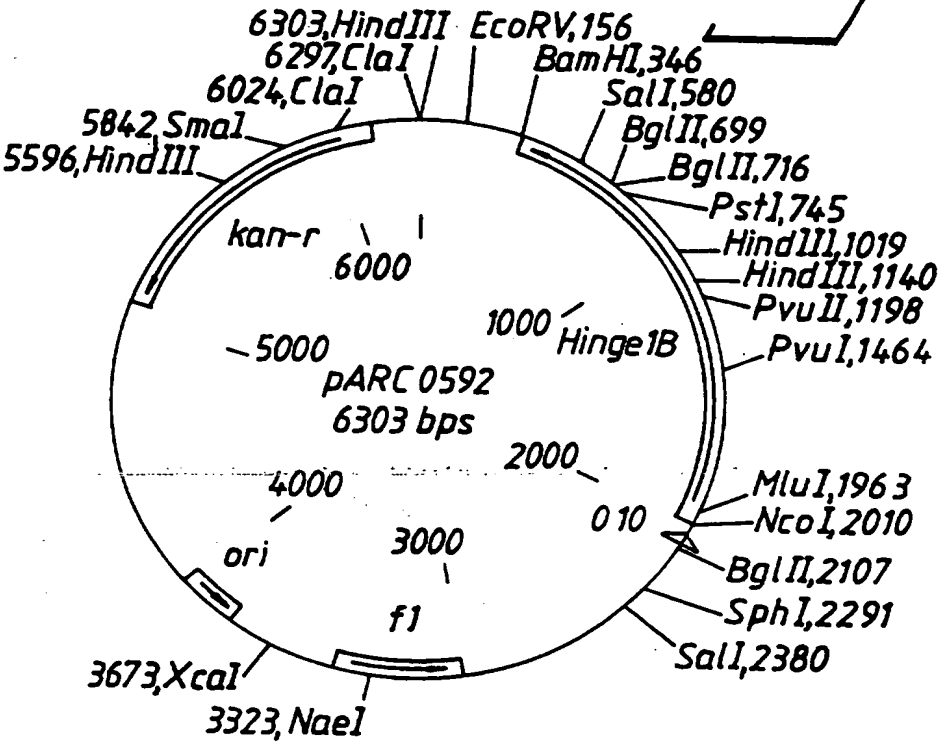

6303,HindIII
EcoRV,156
6297,ClaI
BamHI,346
6024,ClaI
SalI,580
5842,SmaI
BglII,699
5596,HindIII
BglII,716
PstI,745
kan-r
HindIII,1019
6000
HindIII,1140
PvuII,1198
1000
Hinge1B
5000
PvuI,1464
pARC 0592
6303 bps
2000
4000
MluI,1963
010
NcoI,2010
ori
3000
BglII,2107
f1
SphI,2291
SalI,2380
3673,XcaI
3323,NaeI

Fig. 22

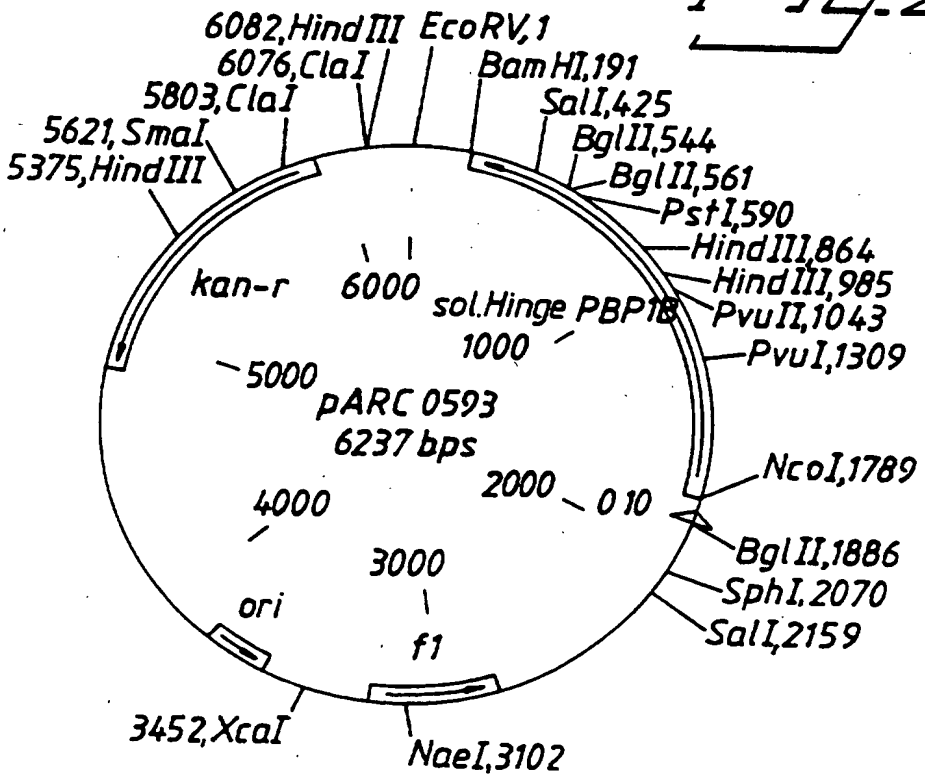

6082,HindIII
EcoRV,1
6076,ClaI
BamHI,191
5803,ClaI
SalI,425
5621,SmaI
BglII,544
5375,HindIII
BglII,561
PstI,590
HindIII,864
kan-r
6000
HindIII,985
sol.Hinge PBP1B
PvuII,1043
1000
PvuI,1309
5000
pARC 0593
6237 bps
NcoI,1789
2000
010
4000
BglII,1886
3000
SphI,2070
ori
f1
SalI,2159
3452,XcaI
NaeI,3102

Fig. 3

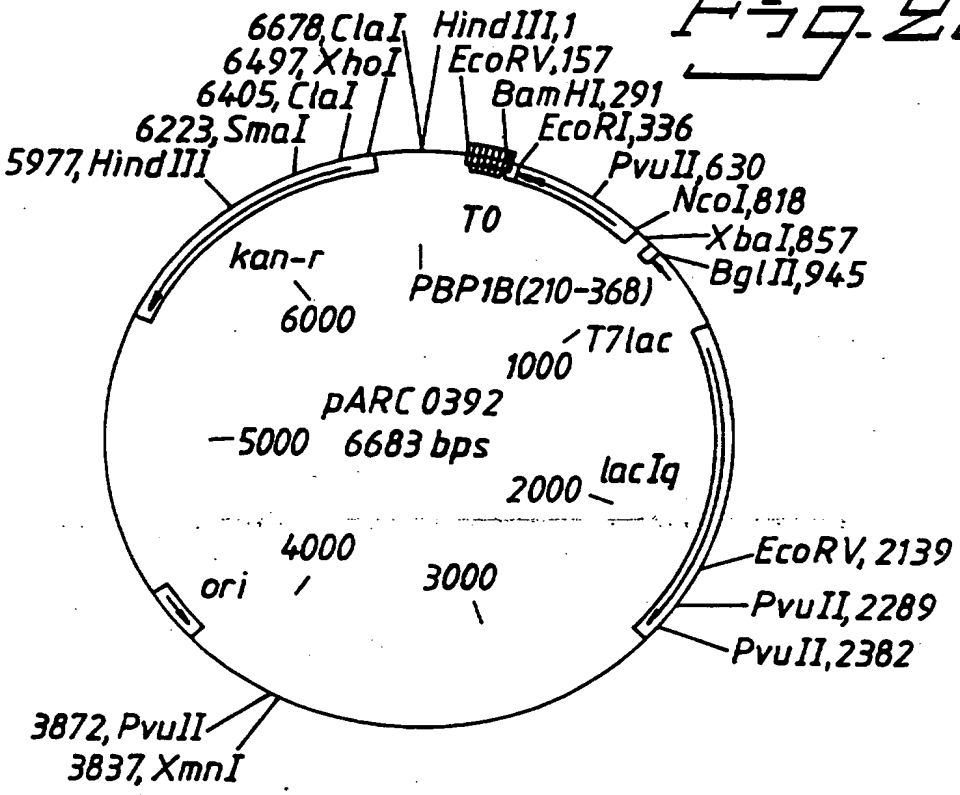

6678, ClaI
HindIII,1
6497, XhoI
EcoRV,157
6405, ClaI
BamHI,291
6223, SmaI
EcoRI,336
5977, HindIII
PvuII,630
NcoI,818
T0
XbaI,857
kan-r
BglII,945
PBP1B(210-368)
6000
T7lac
1000
pARC 0392
5000
6683 bps
lacIq
2000
4000
EcoRV, 2139
ori
3000
PvuII,2289
PvuII,2382
3872, PvuII
3837, XmnI

Fig. 4

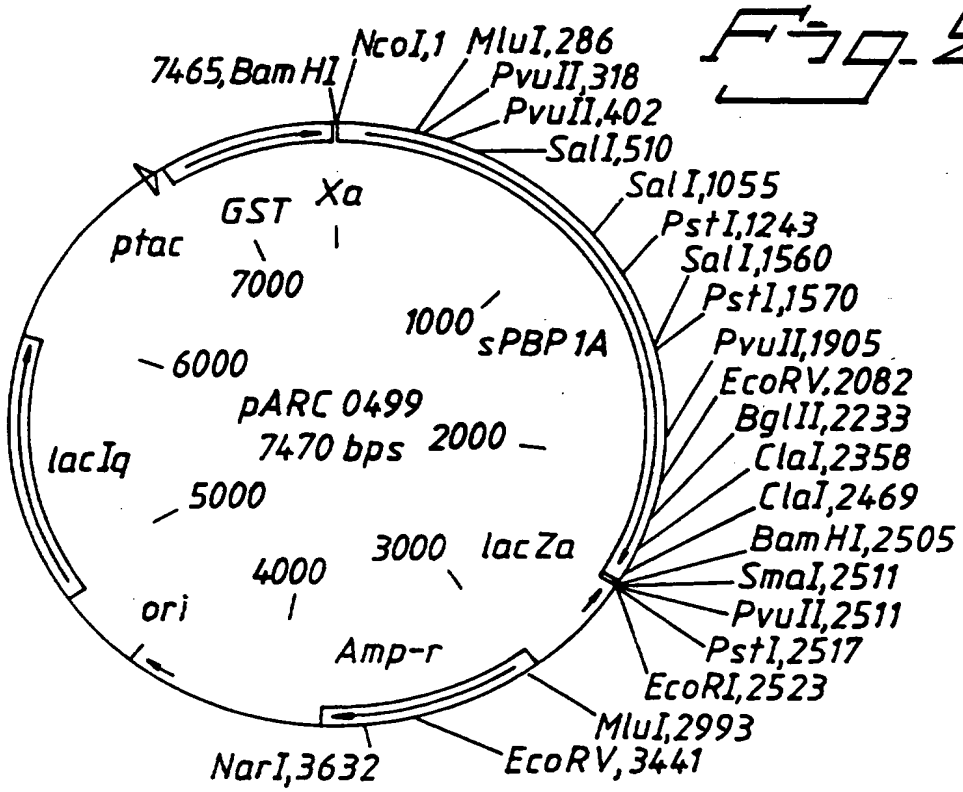
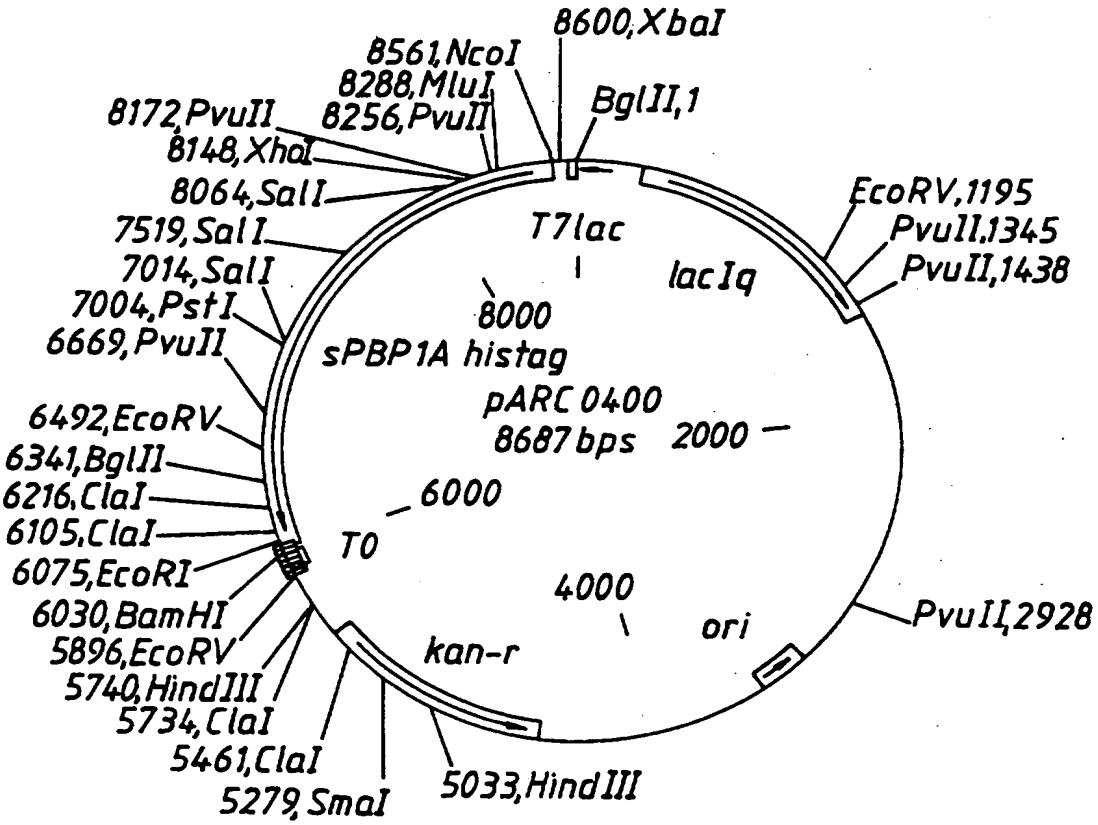

NcoI,1
MluI,286
7465, BamHI
PvuII,318
PvuII,402
SalI,510
SalI,1055
GST
Xa
PstI,1243
ptac
SalI,1560
7000
PstI,1570
1000
sPBP 1A
PvuII,1905
6000
EcoRV,2082
pARC 0499
BglII,2233
7470 bps
2000
ClaI,2358
lacIq
ClaI,2469
5000
BamHI,2505
3000
lacZa
SmaI,2511
4000
PvuII,2511
ori
PstI,2517
Amp-r
EcoRI,2523
MluI,2993
NarI,3632
EcoRV,3441

Fig. 25


8600,XbaI
8561,NcoI
8288,MluI
8256,PvuII
8172,PvuII
8148,XhoI
8064,SalI
7519,SalI
7014,SalI
7004,PstI
6669,PvuII
6492,EcoRV
6341,BglII
6216,ClaI
6105,ClaI
6075,EcoRI
6030,BamHI
5896,EcoRV
5740,HindIII
5734,ClaI
5461,ClaI
5279,SmaI
5033,HindIII
BglII,1
EcoRV,1195
PvuII,1345
PvuII,1438
PvuII,2928
T7lac
lacIq
8000
sPBP1A histag
pARC 0400
8687 bps
2000
6000
T0
4000
ori
kan-r

INTERNATIONAL SEARCH REPORT

International application N .
PCT/SE 95/00761

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/245, C12N 9/24, C12N 9/52, G01N 33/53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPOC, BIOSIS, MEDLINE, SCISEARCH, PATENT CITATION INDEX, EMBL/GENSEQ/DDBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BACTERILOGY, Volume 172, No 1, January 1990, TANNEKE den BLAAUWEN et al, "Interaction of Monoclonal antibodies with the Enzymatic Domains of Penicillin-BindingProtein 1b of Escherichia coli", page 63 - page 70, the whole document --	17-25
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 8, 1993, Robert A. Nicholas et al, "Penicillin-binding Protein 1B from Escherichia coli Contains a Membrane Association Site in Addition to Its Transmembrane Anchor", page 5632 - page 5641, page 5633, column 2, line 48-51; page 634, column 2, line 64 - page 5635, line 13 --	1-2,6-10, 12-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date f th actual completion of the internati nal search	Date of mailing of the internati nal search rep rt
28 February 1996	28 -02- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimil No. +46 8 666 02 86	Authorized fficer Patrick Andersson Teleph n N . +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00761

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Categ ry*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0505151 A2 (ELI LILLY AND COMPANY), 23 Sept 1992 (23.09.92), page 3, line 1 - line 3	1,26
A		2-25
	--	
A	EUROPEAN JOURNAL OF BIOCHEMISTRY, Volume 147, 1985, Jenny K. BROOME-SMITH et al, "The nucleotide sequences of the ponA and ponB genes encoding penicillin-binding proteins 1A and 1B of Escherichia coli K12" page 437 - page 446	1-26
	--	
A	Dialog Information Services, file 154, MEDLINE, Dialog accession no, 08300977, Medline accession no. 93010977, Martin C et al: "Relatedness of penicillin-resistant Streptococcus pneumoniae isolated in south Africa and Spain"; & EMBO J (ENGLAND) Nov 1992, 11 (11) p3831-6	1-26
	--	
A	Dialog Information Services, file 154, MEDLINE, Dialog accession no. 08187042, Medline accession no. 92325042, Martin C et al: "Nucleotide sequences of genes encoding penicillin-binding proteins from Streptococcus pneumoniae and Streptococcus oralis with high homology to Escherichia coli penicillin-binding proteins 1a and 1b"; & J Bacteriol (UNITED STATES) Jul 1992, 174 (13) p4517-23	1-26
	--	

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/02/96

International application No.
PCT/SE 95/00761

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0505151	23/09/92	NONE	

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